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Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B_{12} deficiency – What have we learned?

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ABSTRACT

Background: Maternal vitamin B_{12} (Cbl) deficiency causes nutritional Cbl deficiency in breastfed infants.

Aims: To analyse clinical presentation and metabolic consequences in 40 breastfed infants with Cbl deficiency.

Methods: Cbl levels in serum and breast milk were determined by an electrochemiluminescence immunoassay, methylmalonic acid level by GC/MS, plasma homocysteine by HPLC and propionylcarnitine by MS/MS. Profound Cbl deficiency was found in 17 children (69 ± 17 ng/l, controls 200–900), and milder Cbl deficiency in 23 children (167 ± 40 ng/l). Maternal Cbl deficiency was mostly caused by insufficient Cbl absorption. Only six mothers were vegetarian.

Results: The average age at diagnosis was 4.4 ± 2.5 months. Clinical symptoms included failure to thrive (48% of children), hypotonia (40%), developmental delay (38%) and microcephaly (23%). 63% of children had anaemia (megaloblastic in 28% of all children). All but one patient had methylmalonic aciduria, 80% of patients had hyperhomocysteinemia and 87% had increased aminotransferases. Propionylcarnitine was elevated in two out of 25 infants. Comparing groups with severe and mild Cbl deficiency, a marked difference was found in severity of clinical and laboratory changes.

Conclusion: Maternal Cbl status and diagnostic delay are the major factors influencing severity and progression of Cbl deficiency in breastfed infants. In our cohort, propionylcarnitine was not sufficiently sensitive marker of Cbl deficiency. Although symptoms are reversible on Cbl substitution, permanent neurological damage can result. Selective screening for Cbl deficiency is indicated in all breastfed infants with failure to thrive,

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotranferase; Cbl, Vitamin B_{12} (cobalamin); Hb, Hemoglobin; MCV, Mean cell volume; P-HCY, Plasma homocysteine; SD, Standard deviation; U-MMA, Urinary methylmalonic acid.

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hypotonia, developmental delay, microcephaly or megaloblastic anaemia. **The best prevention in future could be the screening of all pregnant women**.

1. Introduction

Vitamin B_{12} (Cbl, cobalamin) has a variety of biological functions but above all it is essential for hematopoiesis and the development of nervous system. Cbl deficiency in adults may present as megaloblastic anaemia, polyneuropathy, subacute combined neurodegeneration of spinal cord, dementia or depression. The clinical impairment of mature nervous system develops slowly in months or years.[1] This contrasts with Cbl deficiency in infants who undergo extensive growth and development of the brain: Cbl deficiency may cause severe impairment in only a few weeks. The most common symptoms include failure to thrive, hypotonia, irritability or lethargy, developmental delay and even regression,[2–5] epilepsy or movement disorder.[6,7] Brain atrophy, delayed myelination,[8] polyneuropathy and abnormal evoked responses[9] were reported. Laboratory findings usually include megaloblastic anaemia, elevated plasma homocysteine (P-HCY) and increased urinary excretion of methylmalonic acid (U-MMA). Cbl supplementation normalizes the hematological and metabolic disturbances, but early treatment is crucial to prevent neurological residuum such as learning or behavioural problems, secondary epilepsy or mental retardation.

Although the nutritional Cbl deficiency in infancy was already described in 1962[10] and several times later e.g[3,6,7,11–18] and recently reviewed by Dror et al.,[19] the general awareness is still not appropriate. Cbl deficiency in infants is mostly nutritional due to low levels of vitamin B_{12} in the milk of their Cbl-deficient but still asymptomatic mothers. Inherited disturbances of Cbl metabolism are rare.[13] Maternal Cbl deficiency may be caused by various gastrointestinal diseases including achlorhydria, *Helicobacter pylori* infection, celiac disease, Crohn disease, pancreatic insufficiency, treatment with proton pump inhibitors[20] or by insufficient Cbl intake in vegetarian diet. Due to active placental Cbl transport *in utero* resulting in fetal serum Cbl levels twice those in maternal serum,[21] even children of Cbl-deficient mothers usually have enough Cbl for adequate prenatal development. However, they are born with lower stores of Cbl[15] and following their depletion, clinical manifestation gradually develops. The time of manifestation and the speed of progression depend on the severity of maternal deficiency.[13]

We analysed clinical presentation and metabolic consequences in 40 breastfed infants with nutritional Cbl deficiency.

2. Patients and methods

Altogether 40 breastfed infants and small toddlers (19 boys and 21 girls) from 40 families with nutritional Cbl deficiency were included in the study, recognized in participating clinics between 2002 and 2006. Over 5000 children were referred to our centres with a suspected metabolic disease during a five-year period (January 2002 - December 2006). Cbl deficiency was diagnosed in 40 children representing approx. 1 % children from this selective screening.

The inclusion criteria were low or borderline Cbl level in serum in breastfed infants, causing high urinary U-MMA excretion and/or high P-HCY concentration, reversible on enteral Cbl substitution.

Failure to thrive was defined as a weight below the 3rd percentile for age or decrease in the percentile rank of 2 major weight parameters in a 6-months period. Feeding difficulties were mainly characterized by a refusal of breast-feeding and a bottle, and by reluctance to solids. Psychomotor delay was defined as a generalized slowing of mental and physical activity and failure to reach basic developmental milestones.

Fasting blood samples were collected into tubes with and without EDTA. Laboratory investigations included the full blood count and a peripheral blood smear. Cbl levels in serum and breast milk were determined by a electrochemiluminescence immunoassay (Cobas, Roche Diagnostics), methylmalonic acid levels by the GC/MS technique, and total plasma homocysteine levels by HPLC with postcolumn fluorescence detection.[22] Propionylcarnitine concentration was analysed in dried blood spots on API 2000 triple quadrupole MS/MS (Applied Biosystems/MDS SCIEX) with TurboIonSprayk interface.[23]

Cbl levels in serum and breast milk were measured by a kit (Cobas, Roche Diagnostics, USA). According to the manufacturer, the normal range of Cbl levels in serum is 200–900 ng/l in healthy adult men and women. We tested 20 healthy ''disease-free infants'' and results were within the normal range.

3. Ethics

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and approved by the Ethical Committee at the Faculty Hospital. An informed consent was obtained from the parents.

4. Statistical analysis

Statgraphics Plus version 6.0 was used for statistical analyses. Simple linear regression was used to test for the correlation between serum Cbl levels and P-HCY or U-MMA concentrations in infants and between Cbl levels in infants and their mothers. Differences were regarded significant at a $p < 0.05$. Anthropometric data are presented in percentiles for the specific sex and age groups. Values were averaged, and $\pm$2SD values are given.