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Maternal vitamin B_{12} deficiency detected in expanded newborn screening

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ABSTRACT

Objectives: Besides the inherited form, vitamin B_{12} deficiency may be due to diet restrictions or abnormal absorption. The spread of newborn screening programs worldwide has pointed out that non-inherited conditions are mainly secondary to a maternal deficiency. The aim of our work was to study seven cases of acquired vitamin B_{12} deficiency detected during our newborn screening project. Moreover, we aimed to evaluate vitamin B_{12} and related biochemical parameters status on delivering female to verify the consequences on newborns of eventually altered parameters.

Design and methods: 35,000 newborns were screened; those showing altered propionyl carnitine (C3) underwent second-tier test for methylmalonic acid (MMA) on dried blood spot (DBS). Subsequently, newborns positive to the presence of MMA on DBS and their respective mothers underwent further tests: serum vitamin B_{12}, holo-transcobalamin (Holo-TC), folate and homocysteine; newborns were also tested for urinary MMA content. A control study was conducted on 203 females that were tested for the same parameters when admitted to hospital for delivery.

Results: Approximately 10% of the examined newborns showed altered C3. Among these, seven cases of acquired vitamin B_{12} deficiency were identified (70% of the MMA-positive cases). Moreover, our data show a high frequency of vitamin B_{12} deficiency in delivering female (approximately 48% of examined pregnants).

Conclusions: We suggest to monitor vitamin B_{12} and Holo-TC until delivery and to reconsider the reference interval of vitamin B_{12} for a better identification of cases at risk. Finally, newborns from mothers with low or borderline levels of vitamin B_{12} should undergo second-tier test for MMA; in the presence of MMA they should be supplemented with vitamin B_{12} to prevent adverse effects related to vitamin B_{12} deficiency.

Introduction

The methylation of homocysteine to methionine (through the conversion of the group donor methyltetrahydrofolate, MTHF, to tetrahydrofolate, THF) and the reversible rearrangement of methylmalonyl-CoA to succinyl-CoA are two metabolic reactions whose correct functioning is strictly linked to vitamin B_{12} status (Fig. 1). Such vitamin, transformed in cobalamin (adenosylcobalamin, AdoCbl and methylcobalamine, MeCbl), in fact, acts as a cofactor for the enzymes, methionine synthase and L-methylmalonyl-CoA mutase, respectively responsible for the catalysis of the previously reported reactions [1]. When the cobalamin is deficient, an accumulation of the precursors of the mentioned reactions occurs with a deep impact on DNA synthesis, blood red cell production and regeneration and central nervous system development [1].

Besides the inherited form, vitamin B_{12} deficiency may be due to diet restrictions or abnormal absorption [2]. Since the body is unable to synthesize vitamin B_{12}, it must be introduced through diet and specifically through the consumption of animal products as meat, egg, fish, and milk, which are the only aliments containing such vitamin. As a consequence, strictly vegetarian or even vegan individual who do not consume these foods show insufficient intake and therefore low stores of vitamin B_{12} [3,4]; their newborns often show elevated MMA levels [5,6]. Moreover, decreased intake of vitamin B_{12} can also occur because of unrecognized pernicious anemia [7], previous gastric bypass surgery or short gut syndrome [2]. Despite the cause of the vitamin B_{12} deficiency, this condition leads to important consequences on neurological development of fetus in case of pregnancy.

Abbreviations: DBS, dried blood spot; Holo-TC, holo-transcobalamin; Hcy, homocysteine; MMA, methylmalonic acid; NBS, newborn screening; C3, propionyl carnitine; cbl, cobalamin; THF, tetrahydrofolate; MTHF, methyltetrahydrofolate.

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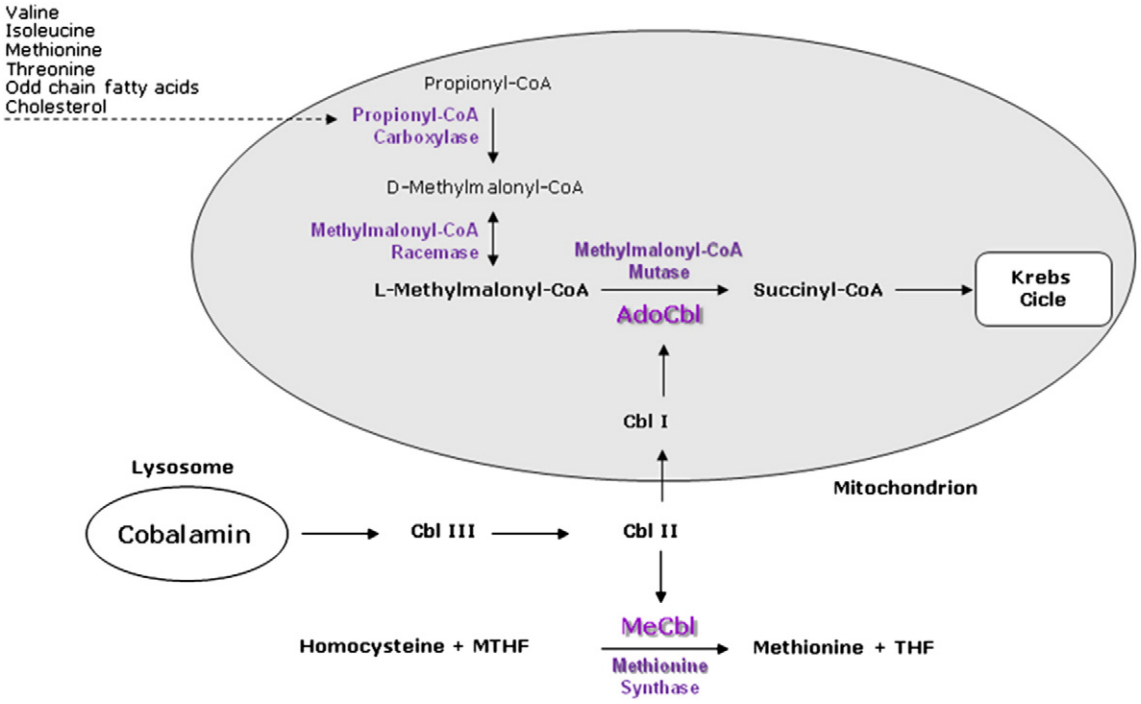

Fig. 1. Methylmalonyl-CoA metabolism and relationship between MMA, homocysteine and vitamin B_{12}. The propionyl-CoA carboxylase converts propionyl-CoA into D-methylmalonyl-CoA that is then racemized into L-methylmalonyl-CoA and isomerized into succinyl-CoA, a Krebs cycle intermediate. The L-methylmalonyl-CoA mutase reaction requires adenosylcobalamin, an activated form of cobalamin (vitamin B_{12}). The methionine synthetase converts homocysteine into methionine. This enzyme requires methylcobalamine as co-factor. The formation of adenosylcobalamin and methylcobalamine depends by intracellular metabolism of cobalamin (Cbl).

The spread of newborn screening (NBS) programs worldwide has greatly contributed to highlight the deep incidence of cobalamin deficiency even in industrialized countries, thus suggesting that vitamin B_{12} deficiency is more common than previously thought and pointed out that non-inherited conditions are mainly secondary to maternal deficiency [7–9].

Newborns, from mothers with a deficiency of vitamin $B_{12,}$ were demonstrated to be 5 times more exposed to the risk of developing neural tube defects [10].

In addition, the higher incidence of infertility, spontaneous abortion, preterm and low weight newborn were shown to be associated to vitamin B_{12} deficiency, confirming the importance of multivitamin and, mostly, vitamin B_{12} supplementation for women [11].

Blood concentrations of propionyl carnitine (C3), arising from the excess of propionyl-CoA, homocysteine (Hcy) and methylmalonic acid (MMA) increase in cobalamin deficiency; the accumulation of these metabolites anticipates hematological and neurological signs. MMA is usually considered the most sensitive marker; accordingly, during the last years, urine and plasma methylmalonic acid levels have been used to better adjust therapy during pregnancy in women affected by cobalamin A disease [12]. Indeed, early detection of vitamin B_{12} deficiency is crucial for a prompt treatement based on the administration of the deficient vitamin; in fact, manifestations of vitamin B_{12} deficiency (development delay, weakness, failure to thrive) are often non-specific with a consequent high risk of delayed diagnosis.

Here we report a description of seven cases of acquired vitamin B_{12} deficiency detected during our six years of newborn screening pilot project. A control study was also conducted on healthy 203 females and their newborns when admitted to hospital for delivery.

Materials and methods

Biochemical analysis

Amino acids and acylcarnitine analysis on DBS were performed on an API 2000 Triple–Quadrupole Mass Spectrometer (Applied Biosystems/SCIEX, Toronto, Canada) coupled with the Agilent high performance liquid chromatograph of the 1100 series (Agilent Technologies, Waldbronn, Germany) as described [13]. Methylmalonic acid (MMA) was measured on dried blood spot (DBS) as described [14]; MMA was extracted from a 3.2 mm DBS and the chromatographic separation of the extract was performed on a C6-phenyl column. The extraction and quantification of MMA from urine were performed as already reported using a GC–MS Agilent 5975C apparatus equipped with an HP-5MS column [13]. Vitamin B_{12}, holo-transcobalamin (Holo-TC, the active form of cobalamin), homocysteine (Hcy) and folates were measured on serum by Architect System Kits (Abbott, Illinois, www.abbottdiagnostics.com). All the used reference intervals are not age-specific and arose from the laboratory experience.

CVs for concentrations in the range of the low, medium and high controls, for serum vitamin B12, Holo-TC, homocysteine and folates were $\leq$10%, $\leq$8%, $\leq$10% and $\leq$11%, respectively. Sensitivity of each biochemical assay, as defined by Limit of Detection (LoD) and Limit of Quantitation (LoQ) was the following: vitamin B12 (LoD: 88 pmol/L; LoQ: 93 pmol/L); Holo-TC (LoD: 2.1p mol/L; LoQ: <3p mol/L); homocysteine (LoD: 0.72 μM; LoQ: $\leq$1 μM); folates (LoD: 0.95 nmol/L; LoQ: 3 nmol/L). Biochemical method performance for MMA determination on DBS was as previously reported [14].

Patients and controls/study design

During six years about 35,000 newborns were screened for inborn errors of metabolism and, among them, all those showing on DBS a propionyl carnitine (C3) value higher than 3.16 μmol/L, with or without altered C3/C2 (acetyl carnitine) (n.v. < 0.18) and/or C3/C16 (palmitoyl carnitine) (n.v. <1.80) ratios, underwent second-tier test for the detection of MMA on dried blood spot. Subsequently, newborns positive to the presence of MMA on DBS and their respective mothers underwent further laboratory testing: serum vitamin B_{12}, Holo-TC, folate and homocysteine; newborns were also tested for urinary MMA content.

A control study was then conducted on 203 females who were tested for serum vitamin B_{12}, Holo-TC, folate and homocysteine when admitted