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Elevated dimethylglycine in blood of children with congenital heart defects and their mothers

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ABSTRACT

Objective. Congenital Heart Defects (CHD) may be related to nutritional deficiencies affecting the methylation cycle. We aimed to study the metabolic markers of the betaine homocysteine methyl transferase (BHMT) pathway in children with CHD and their mothers compared to children without CHD and their mothers.

Materials and Methods. Children with CHD (n=105, age < 3 years) and mothers of 80 of the affected children were studied. The controls were non-CHDs children of comparable age as the CHD group (n=52) and their mothers (n=50). We measured serum or plasma concentrations of the metabolites of the methylation cycle homocysteine (HCY), methylmalonic acid (MMA), cystathionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), betaine, choline, and dimethylglycine (DMG).

Results. Children with CHD had higher plasma SAM (131 vs. 100 nmol/L) and DMG (8.7 vs. 6.0 μmol/L) and lower betaine/DMG ratio (7.5 vs. 10.2) compared to the controls. Mothers of CHD children showed also higher DMG (6.1 vs. 4.1 μmol/L) and lower betaine/DMG ratio compared with the mothers of the controls. Higher SAM levels were related to higher cystathionine, MMA, betaine, choline, and DMG. MMA elevation in the patients was related to higher HCY, SAM, betaine and DMG.

Conclusions. Elevated DMG in CHD children and their mothers compared to the controls can indicate upregulation of the BHMT pathway in this disease group. Nutritional factors are related to metabolic imbalance during pregnancy that may be related to worse birth outcome.

1. Introduction

Heart morphogenesis is a complex process requiring the coordination of cellular differentiation, migration, proliferation and apoptosis. Congenital heart defects (CHDs) are the most common birth defects [1,2]. Approximately 15% of CHD can be attributed to known risk factors [3]. The remaining CHDs are thought to result from factors affecting the intrauterine environment during gestation including environmental factors, maternal lifestyle, and both maternal and fetal genetic susceptibilities.

Abbreviations: CHD, congenital heart defects; HCY, homocysteine; MMA, methylmalonic acid; DMG, dimethylglycine; BHMT, betaine homocysteine methyl transferase; UPLC-MS/MS, ultra performance liquid chromatography; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine. MAT, L-methionine S-adenosyltransferase; CBS, cystathionine beta synthase.

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Maternal dietary or environmental factors can affect maternal DNA-methylation and that of the offspring [4,5]. Several birth defects have been related to changes in methylation [6–8]. DNA methylation at CpG rich sites and the histone methylation are mediated by specific S-adenosylmethionine (SAM)-dependent methyltransferases. The methylation during embryogenesis comprises a key step for all subsequent cascades of events [9,10] and ensures synthesis of carnitine, polyamines and other methylated substrates.

The availability of the methyl groups is influenced by several nutrients like folate, methionine, vitamin B12, betaine and choline. Folate and vitamin B12 are required for the remethylation of homocysteine (HCY) to methionine. S-adenosylmethionine is synthesized from methionine and represents the primary methyl donor for numerous cellular reactions. After methyl transfer, SAM is converted into S-adenosylhomocysteine (SAH). Hyperhomocysteinemia is associated with elevated SAH [11], the potent inhibitor of cellular methyltransferases.

The betaine homocysteine methyltransferase (BHMT) pathway is an alternative source for the methyl group. In this pathway, the methyl group is transferred from betaine to HCY, forming dimethylglycine (DMG) and methionine. This pathway contributes 50% of the HCY-methylation capacity of the liver [12]. This route is important in pregnancy [13] particularly in cases with folate or B12 insufficiency [14]. In the mitochondria, DMG is converted into sarcosine and further to glycine by two oxidative demethylation steps mediated by DMG dehydrogenase and sarcosine dehydrogenase, respectively. The active one-carbon group formed via DMG is used preferentially for the formation of serine from sarcosine [15]. Choline is an important nutrient and a precursor for betaine. Animal studies have shown that defects in choline metabolism are related to fetal death or severe neurolization defects [16]. Furthermore, severe heart defects were observed when a choline deficient diet (1/8 of the recommended daily intake) was administered 6 weeks before conception to mice [17]. Choline may have an important role in birth defects, at least partly by providing methyl groups.

Multivitamins containing folic acid before and throughout the first trimester can reduce the risk of having a child with CHD [18–20]. Moreover, many women with adverse pregnancy outcomes, including those with CHD births have elevated concentrations of HCY [21,22]. Abnormal methylation was also reported in children affected with CHDs [23]. However, the metabolites of the BHMT pathway have not been investigated in relation to CHD. The aim of the current study was to determine whether biomarkers of the methyl cycle, especially those related to the BHMT pathway are different between children with CHD and their mothers compared with healthy children and their mothers. The role of betaine and choline as methyl donors is studied in a population of a high prevalence of vitamin B12 deficiency.

2. Subjects and methods

2.1. Subjects

Patients with CHD and their mothers were recruited from the University Hospital of Damascus, the Pediatrics' University Hospital, and the Heart Surgery University Hospital. The controls were recruited from the nursery of the Paediatrics University Hospital of Damascus. The recruitment phase was between August 2010 and June 2011.

The study included CHD children (n=105) and 80 mothers of the CHD group. All types of CHD were included (ventricular septal defects, atrioventricular septal defects, transposition of the great arteries, coarctation of the aorta, pulmonary valve stenosis, tetralogy of Fallot, pentology of Fallot). The age of the CHD children was below 3 years and the affected pregnancy was within the last 3 years. The controls were non-CHDs children with comparable age as the CHD group (n=52) and their mothers (n=50). Exclusion criteria were, all chromosomal defects (including Down syndrome) and other birth defects, recent operations, and kidney or hepatic diseases. Exclusion criteria for the mothers were current pregnancy, diabetes mellitus before the CHD child, and recent operations. All mothers were apparently healthy. None of the children or the mothers was taking vitamin supplements at the time of the study.

A standardized interview and questionnaire were completed for each mother. The complete medical history of the child and the mother, current medications, maternal health condition during the affected pregnancy, and co-morbidities were documented. All children with CHD were diagnosed by heart echocardiography performed by a cardiologist pediatrician. The defect phenotype was documented. The study was approved by the ethical committee of Damascus University Hospital, and all participants signed a written consent form. The study was performed in adherence with the guidelines of the Declaration of Helsinki.

2.2. Blood sampling and biochemical measurements

Venous blood (7 ml) was collected into dry tubes and those containing K^+EDTA. K^+EDTA tubes were chilled on ice and centrifuged within 40 min. Several aliquots were prepared and stored at −70 °C. A volume of 50 μl of 1 N acetic acid was immediately added to 500 μl of EDTA plasma and kept at −70 °C for SAM and SAH assays. Total blood count was immediately measured in the laboratories of the study sites.

The plasma concentrations of betaine, choline, and DMG were measured with a stable-isotope dilution UPLC-MS/MS method (Waters, Milford, MA, USA) [24]. The between-day CVs for betaine, choline were <8%, and for DMG, CVs were<5%. The serum concentrations of HCY, methylmalonic acid (MMA is a marker for B12 status), and cystathionine (a marker for the transsulfuration pathway) were measured by gas chromatography–mass spectrometry (Agilent Technologies, Santa Clara, California, USA) as described by Stabler et al. [25]. The coefficient of variations (CVs) % for the MMA assay were <2.5% and for HCY and cystathionine assays, the CVs were<4%. The plasma concentrations of SAM and SAH were measured by UPLC-MS/MS (Waters, Milford, MA, USA) as described by Kirsch et al. [26]. The CVs % for the SAH and SAM assays were <5%. The concentration of holotranscobalamin (holoTC) (a marker for vitamin B12 status) was measured in a subset of samples (n=86) to verify MMA elevation. HoloTC was measured using a specific monoclonal antibody against holoTC, and detection was performed using alkaline

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