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

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ARTICLE INFO

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 dimethylglycine (DMG).

MMA elevation in the patients was related to higher HCY, SAM, betaine, 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.

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Keywords: Congenital heart defects, Betaine, Dimethylglycine, Methylation

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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http://dx.doi.org/10.1016/j.metabol.2013.01.024
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 pregnancy to achieve an adequate 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.

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) assay for betaine, choline were <8%, and for DMG, CVs were <5%. The serum concentrations of HCY, methylmalonic acid (MMA) assay for betaine, choline were <8%, and for DMG, CVs were <5%

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.

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The plasma concentrations of SAM and SAH were measured by UPLC-MS/MS (Waters, Milford, MA, USA) as described by Kirsch 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

...
phosphatase-labeled anti-holotranscobalamin (AxSYM, Abbott, Germany). The concentrations of serum folate were measured only in maternal samples using immunoassay (Elecsys 2010; Roche, Mannheim, Germany).

The statistical analyses were performed with SPSS (version 19.0). Results are shown as mean (SD, standard deviation). Means or medians continuous variables were compared between two independent groups using ANOVA or Mann–Whitney tests, respectively. Chi-square test was applied to compare categorical variables. Tertiles of child plasma concentrations of SAM were compared for all other continuous variables using ANOVA test followed by Tamhane-T test adjustment. Stepwise, multiple backward regression analysis was applied to identify significant predictors of child SAM. P values below 0.05 were considered statistically significant.

3. Results

Table 1 shows the main characteristics of the study population. CHD and control children had similar ages and gender distribution (Table 1). Mean child haemoglobin was higher (p=0.002) and MCV tended to be higher (p=0.087) in CHD patients compared to the controls. Maternal haemoglobin tended to be higher in mothers of CHD patients compared to mothers of the controls (p=0.095). Mothers of the control children were older than mothers of the CHD children (30.3 vs. 27.3 years; p=0.004). Mothers of the CHD children had lower household income, lower educational status and higher parity births than mothers of the controls (Table 1). No differences were found in BMI, usage of vitamin supplements during the first gestation trimester, or the incidence of previous abortion.

Table 2 shows CHD phenotypes in the patients. Forty eight children had single lesion and 57 had multiple lesions that ranged from moderate to complex defects. Patients with a single lesion and those with multiple lesions showed no significant differences in any of the biomarkers tested (results not shown).

The concentrations of the main biomarkers in the children and the mothers are depicted in Table 3. Compared to the control children, children with CHD showed higher levels of SAM (mean 100 vs. 131 nmol/L; p<0.001) and DMG (6.0 vs. 8.7 μmol/L; p=0.007) and lower betaine/DMG ratio (10.2 vs. 7.5;
p<0.001), but comparable SAM/SAH ratio (5.5 vs. 5.7: p=0.357). The concentrations of cystathionine (p=0.078) and those of MMA (p=0.089) tended to be higher, and the concentrations of betaine (p=0.078) tended to be lower in the CHD group compared with the controls (Table 3). All other markers were not different between the two groups (Table 3). Compared to mothers of the controls, mothers of children with CHD showed higher concentrations of DMG (6.1 vs. 4.1; p=0.010), and lower concentrations of SAH (16.8 vs. 20.8; p=0.028) and betaine/DMG ratio (10.5 vs. 14.6; p=0.001). The concentrations of SAM, MMA, folate, HCY, cystathionine, and betaine were not different between the two groups (Table 3).

Patients with CHD were stratified according to their SAM level (Table 4). Tertiles of SAM were compared for other markers in the CHD children and the mothers (Table 4). CHD children with higher SAM were younger, and they had higher SAM/SAH ratio (p<0.001), cystathionine (p=0.033), MMA (p=0.002), betaine (p=0.018), choline (p=0.001), and DMG (p<0.001). The concentrations of maternal plasma DMG were also higher in mothers of CHD-children (p=0.03) in the third SAM tertile, compared to those in the lowest SAM tertile (Table 4). Although betaine was not significantly different between the mothers according to child SAM tertiles, the ratio of betaine/DMG tended to be lower at higher SAM (p=0.092). The concentrations of maternal SAM tended to be higher in the third tertile of child SAM compared to the first tertile (p=0.067). All other maternal markers did not differ according to child SAM.

Table 4 – Maternal and child blood biomarkers according to SAM tertiles in children with CHD.

<table>
<thead>
<tr>
<th>SAM tertiles in CHD children</th>
<th>p-value</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
<td>Middle</td>
<td>Highest</td>
</tr>
<tr>
<td>Children with CHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, months</td>
<td>19.6 (14.8)</td>
<td>18.6 (9.9)</td>
</tr>
<tr>
<td>HCY, μmol/L</td>
<td>10.2 (4.6)</td>
<td>9.3 (3.7)</td>
</tr>
<tr>
<td>Cystathionine, nmol/L</td>
<td>359 (264)</td>
<td>328 (174)</td>
</tr>
<tr>
<td>SAM, nmol/L</td>
<td>90 (22)</td>
<td>124 (11)</td>
</tr>
<tr>
<td>SAH, nmol/L</td>
<td>25.9 (12.5)</td>
<td>22.9 (8.7)</td>
</tr>
<tr>
<td>SAM/SAH ratio</td>
<td>4.3 (2.0)</td>
<td>6.0 (1.9)</td>
</tr>
<tr>
<td>MMA, nmol/L</td>
<td>511 (435)</td>
<td>588 (362)</td>
</tr>
<tr>
<td>Betaine, μmol/L</td>
<td>43.1 (17.5)</td>
<td>58.7 (31.8)</td>
</tr>
<tr>
<td>Choline, μmol/L</td>
<td>13.7 (7.7)</td>
<td>15.1 (4.1)</td>
</tr>
<tr>
<td>DMG, μmol/L</td>
<td>5.9 (3.2)</td>
<td>8.0 (4.0)</td>
</tr>
<tr>
<td>Betaine/DMG ratio</td>
<td>8.6 (4.6)</td>
<td>7.6 (2.9)</td>
</tr>
<tr>
<td>Mothers of the CHD patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>27.3 (5.5)</td>
<td>26.4 (5.3)</td>
</tr>
<tr>
<td>HCY, μmol/L</td>
<td>11.6 (3.9)</td>
<td>12.8 (4.6)</td>
</tr>
<tr>
<td>Cystathionine, nmol/L</td>
<td>165 (110)</td>
<td>187 (50)</td>
</tr>
<tr>
<td>SAM, nmol/L</td>
<td>78 (25)</td>
<td>85 (16)</td>
</tr>
<tr>
<td>SAH, nmol/L</td>
<td>25.9 (11.6)</td>
<td>22.9 (8.7)</td>
</tr>
<tr>
<td>SAM/SAH ratio</td>
<td>5.2 (2.6)</td>
<td>5.6 (1.7)</td>
</tr>
<tr>
<td>MMA, nmol/L</td>
<td>329 (263)</td>
<td>501 (463)</td>
</tr>
<tr>
<td>Betaine, μmol/L</td>
<td>5.9 (3.2)</td>
<td>8.0 (4.0)</td>
</tr>
<tr>
<td>Choline, μmol/L</td>
<td>13.7 (7.7)</td>
<td>15.1 (4.1)</td>
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<tr>
<td>DMG, μmol/L</td>
<td>5.9 (3.2)</td>
<td>8.0 (4.0)</td>
</tr>
<tr>
<td>Betaine/DMG ratio</td>
<td>8.6 (4.6)</td>
<td>7.6 (2.9)</td>
</tr>
</tbody>
</table>

Data are means (SD). P values are according to ANOVA test applied on the log-transformed data.

Table 5 – The concentration of the metabolites according to serum MMA in children with CHD.

<table>
<thead>
<tr>
<th>MMA in CHD children, nmol/L</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>&lt; Median</td>
</tr>
<tr>
<td>MMA, nmol/L</td>
<td>323 (95)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>[148-481]</td>
</tr>
<tr>
<td>[Range]</td>
<td>8.1 (2.8)</td>
</tr>
<tr>
<td>HCY, μmol/L</td>
<td>323 (242)</td>
</tr>
<tr>
<td>Cystathionine, nmol/L</td>
<td>114 (35)</td>
</tr>
<tr>
<td>SAM, nmol/L</td>
<td>25.9 (11.6)</td>
</tr>
<tr>
<td>SAH, nmol/L</td>
<td>5.1 (2.3)</td>
</tr>
<tr>
<td>SAM/SAH ratio</td>
<td>49.3 (24.2)</td>
</tr>
<tr>
<td>Betaine, μmol/L</td>
<td>15.6 (5.2)</td>
</tr>
<tr>
<td>Choline, μmol/L</td>
<td>7.5 (4.9)</td>
</tr>
<tr>
<td>DMG, μmol/L</td>
<td>7.6 (3.5)</td>
</tr>
</tbody>
</table>

Data are means (SD). P values are according to ANOVA test.

MMA was divided by median MMA (482 nmol/L) in the CHD group.

Table 6 – Predictors of child SAM (dependent variable).

<table>
<thead>
<tr>
<th>Independent variables entered (only child variables)</th>
<th>Variables with significant effects</th>
<th>P</th>
<th>Regression coefficient (beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, DMG, SAH, MMA, HCY, cystathionine, betaine, choline</td>
<td>Age</td>
<td>0.006</td>
<td>-29.9</td>
</tr>
<tr>
<td></td>
<td>MMA, HCY</td>
<td>0.002</td>
<td>+48.2</td>
</tr>
<tr>
<td></td>
<td>cystathionine, MMA</td>
<td>&lt;0.001</td>
<td>-193.7</td>
</tr>
<tr>
<td></td>
<td>betaine, choline</td>
<td>0.006</td>
<td>+54.7</td>
</tr>
<tr>
<td></td>
<td>DMG</td>
<td>&lt;0.001</td>
<td>+92.9</td>
</tr>
</tbody>
</table>

R-square=0.64. Backward regression, applied on the log-transformed data. Constant=0.918.

Fig. 1 – The correlation between MMA and HCY in all children (with and without CHD) and all mothers. The correlation coefficients are according to Spearman test.
Because vitamin B12 deficiency is common in this population, we divided the CHD patients by median child MMA (Table 5). CHD children with higher MMA had higher HCY, SAM, betaine, cystathionine, and DMG compared to children with lower MMA. The concentrations of holoTC were available from 24 CHD patients. Higher concentrations of holoTC were found in children with MMA below the median compared to the group with MMA above the median [mean holoTC=68 nmol/L (n=10) vs. 20 nmol/L (n=14); p=0.04].

Backward multiple regression analysis was applied to identify predictors of child SAM in the CHD group. Child DMG, MMA, cystathionine (all positive predictors) and HCY and age (negative predictors) predicted concentrations of SAM in children with CHD (Table 6). The concentrations of MMA and HCY correlated positively in the total group of children and in the mothers (children: r=0.449, p<0.001, and mothers: r=0.255, p=0.007) (Fig. 1).

4. Discussion

Maternal nutrition is an important causal factor related to several birth defects [27–29]. The potential role for choline metabolism in birth defects has been suggested by a few animal studies [16,17], but clinical studies are limited. Multivitamin supplementation or folic acid fortification is related to a metabolism in birth defects has been suggested by a few animal studies. The plasma concentrations of betaine (mean 50.9 μmol/L), choline (mean 11.8 μmol/L) and DMG (4.1 μmol/L) in the current study are markedly higher than those reported in young women from other populations. One study on US women (mean age 29 years) showed that mean plasma concentrations of betaine, choline, and DMG were 25.0, 6.2, and 2.4 μmol/L, respectively [43]. Moreover, in our earlier study on 74 young German women (mean age 35 years), mean plasma concentration of betaine was 11.7 μmol/L, that of choline was 6.7 μmol/L, and DMG was 2.2 μmol/L [24]. The difference in betaine concentrations between US and German women from the two studies is probably related to betaine sparing because of folic acid fortification in the US. There seems to be no differences in the utilization of the BHMT pathway between US and German women since concentrations of DMG are very similar. In contrast, the utilization of the BHMT pathway as a source for the methyl groups seems to be more active in the current study on Syrian women. This can be explained by vitamin B12 deficiency in this population [32,33,44,45] since the BHMT pathway delivers SAM independent on vitamin B12. Moreover, deficiency of other nutrients (such as methionine or vitamin B2) may also affect the regulation of the methylation cycle [17,46].

The current study has few limitations. First, the reason of CHD and their mothers have higher DMG compared to control children and mothers, this may reflect some maternal transmission of this metabolic condition.

DMG and SAM are known to inhibit BHMT activity [41]. Deletion of BHMT gene caused a 43% reduction in hepatic SAM and a 3-fold increase in hepatic SAH concentrations, thus resulting in a severe reduction in methylation potential [42]. Our study showed the opposite condition (30% higher SAM) suggesting a stimulation of the BHMT pathway. Alternatively, elevated SAM and DMG may be related to enhanced MAT (causing low methionine) or decreased dimethylglycine dehydrogenase and sarcosine dehydrogenase activities. Future studies may measure methionine and sarcosine to rule out this possibility.

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The current study has few limitations. First, the reason of the metabolic changes that we found in CHD children (elevated DMG, and SAM) and their mothers (elevated DMG) is not known and a causality link to CHD can not be assumed. Second, the concentrations of other related metabolites like methionine, methylglycine (sarcosine) were not measured in the current study. Finally, oxidative stress might have an impact on the methylation cycle, since both CBS [34,35] and methionine synthase [36] are sensitive to the oxidative balance.

Dimethylglycine dehydrogenase and sarcosine dehydrogenase are mitochondrial folate binding proteins [37]. Both enzymes participate in the respiratory chain system. Mitochondrial disorders have been described in patients with tetralogy of Fallot [38,39]. Furthermore, oxidative stress has been linked to failure of myocardial remodeling [40]. Therefore, elevated DMG suggests a link to mitochondrial dysfunction in CHD. Since DMG was increased in both CHD children and their mothers, this may reflect some maternal transmission of this metabolic condition.

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metabolic profile in CHD children and their mothers may be related to similar genetic background, dietary habits or lifestyle factors related to the disease. The concentrations of betaine and its utilization as a methyl donor via the BHMT pathway were upregulated in this study. Our results strongly suggest that nutritional factors are related to metabolic derangements during pregnancy that might be related to worse birth outcome. The role of the BHMT pathway in the pathogenesis of CHD needs further investigation.

Author contributions

RA: design of the study, recruitments of the participants, sample collection, data interpretation and manuscript writing. FALQ: design of the study, data collection. SS: data collection. JG: data analysis. RO: design of the study, data analysis, data interpretation and manuscript writing.

Funding

The study was partly funded by the University of Damascus.

Acknowledgments

We would like to thank the medical teams at Damascus University Pediatrics’ hospitals for their support in patient’s recruitment. We are thankful to Professor Muhidien Jouma for the thoughtful discussion during the planning phase of the study.

Conflict of interest

The authors have no conflict of interest regarding this article.

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