Research Article

25-OH Vitamin D and ADMA in Relation to C1 Metabolism in Children with Congenital Heart Defects and Their Mothers

Ranwa Alsayed [1*], Faizeh AL-Quobaili[1]
[1]Damascus University, Faculty of Pharmacy, Department of Biochemistry, Syria.
***Corresponding author's E-mail:** ranwasyd@gmail.com

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ABSTRACT

Congenital heart defects (CHD) may be related to nutritional deficiencies affecting the methylation cycle, and thus several metabolic pathways including that of vitamin D (25-OHD), and asymmetric dimethyl arginine ADMA. Children with CHD (age < 3 years) and mothers of the affected children were studied. The controls were non-CHDs children of comparable age as the CHD group and their mothers. We measured Plasma concentrations of; 25-OHD in CHD children (n=70), and mothers (n = 73) and in control children (n = 43), and mothers (n = 43). Asymmetric dimethyl arginine (ADMA) in CHD children (n = 47) and CHD mothers (n = 65), and compared to ADMA of control children (n = 37) and control mothers (n = 28), correlations of the two parameters with other one carbon metabolism parameters are studied. Children with CHD had higher plasma 25-OHD (71.7 vs. 45.1 nmol/L) compared to the controls. Plasma ADMA tended to be lower in CHD children (1.01 vs. 1.92 µmol/L). Mothers of CHD children and those of the controls did not differ in their plasma 25-OHD (20.2 vs. 21.7 nmol/L) nor in their ADMA levels (0.42 vs. 0.47 µmol/L). Plasma 25-OHD levels in the patients, but not in the controls showed a negative correlation to maternal ADMA (r = -0.449, p = 0.004), both parameters in CHD children correlated negatively to child betaine / dimethylglycine methylation ratio BET/DMG respectively for 25-OHD, ADMA (r = -0.266, p = 0.027), (r = -0.358, p = 0.015). Higher 25-OHD in CHD children and its correlation with maternal ADMA and methylation biomarkers may indicate different metabolism of maternal origin for this compound in CHD children, vitamin D metabolism and ADMA metabolism should be further investigated in this group of patients.

Keywords: ADMA, Choline, Congenital heart defects, Methylation, 25 hydroxy vitamin D.

INTRODUCTION

Vitamin D acts as a hormone that regulates calcium and phosphorus homeostasis, modulates the immune system, the cellular differentiation, and apoptosis.[1-2]

Vitamin D deficiency during pregnancy and in neonates is a worldwide problem[3-6], Pregnancy and lactation represent times of increased requirements for vitamin D. however, to date no clear international RDA for pregnant women is available due to the fact that many different factors are contributing in determining the level of the active form in the blood. Severe vitamin D deficiency during pregnancy has been related to pregnancy complications and poor outcome[7-8], and has been linked to cardiac abnormality in studies on animals.[9-10]

The major circulating and storage form of vitamin D, 25-Hydroxy vitamin D (25-OHD), is the direct precursor of the biologically active form 1,25-$(OH)_2$D, 25-OHD is transported to the foetus and should undergo a second hydroxylation by the mitochondrial enzyme cytochrome 1α-OHase or B27 located in the renal cells but also in many other cells. To a lesser degree the active form is also transported, where binding to vitamin D binding protein is a detrimental factor.[11] There is inconsistent evidence regarding the mechanisms involved in transplacental transfer of 1,25-$(OH)_2$D.[12] But circulating maternal levels of 1,25-$(OH)_2$D are approximately 2-fold higher during pregnancy compared to non-pregnant women[13], suggesting enhanced production as a large proportion of maternal 1,25-$(OH)_2$D is derived from decidua and placental cells.[14] Placental epigenetic regulation of vitamin D metabolism has been reported. [15] In contrast, vitamin D has been related to epigenetic modifications. Congenital birth defects could be related to epigenetic events. On the one hand, binding of the activated vitamin D receptor to retinoid X receptor enables effects of transcription factors and methylation on the chromatin.[16-17] On the other hand, aberrant gene methylation has been linked to several adverse birth outcomes.[18-20]

The protein methyl transferase 4 (PRMT4 or Co activator associated methyl transferase 1)) acted as a (steroid receptor coactivator) and plays an essential role in the response to vitamin D signal by methylation of Arginine 17 at histone 3, it has a positive role in gene transcription and translation, this molecule links vitamin D effectiveness to Arginine methylation[21], Asymmetric dimethyl arginine (ADMA) is released during the the turnover of proteins that are methylated on arginine residues. This compound link one carbon metabolism to protein methylation from one side and, arginine methylation to vitamin D effectiveness from the other side; as arginine residues in nuclear and cytoplasmic proteins can be methylated by protein arginine N-methyl transferase (PRMT) family of enzymes which utilize S-Adenosyl methionine (SAM). This was found important for epigenetic programming of early embryos in mouse[23] and, this kind of epigenetic mechanisms provide transcription memory during cell development as proved

on myeloid cells[24]. One possible target for this vitamin D activation and normal Arginine methylation, will be normal embryonic myosin especially that of striated muscles as, this protein contains methylated arginine and vitamin D deficiency causes reduced actomyosin content of myofibrils and low serum levels of muscle enzymes.[25-26]

Recent studies suggested a link between vascular diseases and vitamin D in adults. For example, recent studies on pregnant women have shown an association between vitamin D deficiency and the risk of preeclampsia.[27-28]

ADMA acts as an endogenous inhibitor of nitric acid oxide synthase (NOS), which competes with L-Arginine for binding to NOS[29], and gained recently attention as a cardiovascular marker.[29-31] While its role as a preeclamptic factor remain to be clearly defined.[32-33] Reporeter & Corbin[34] identified ADMA in the hydrolysate of myosin derived from embryonic muscle or cultured myoblast, Suggesting a role for ADMA in this embryonic tissue.

The present study was undertaken to investigate 25-OHD and ADMA status in children with CHD and their mothers, compared to children without CHD and their mothers. The study examined the relationship between vitamin D, ADMA and one carbon metabolism markers of folate and methionine cycles, as their metabolic pathways are intersected and they have been related to pregnancy development and outcome.

MATERIALS AND METHODS

Subjects

The study population has been described in an earlier report.[35] Children 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 took place between August 2010 and June 2011 for both groups. The study included CHD children (age < 3 years) and their mothers of the CHD group the exact number included for each test is mentioned in the tables 1-4. All types of CHD were included. The controls were non-CHDs children with comparable age as the CHD group and their mothers, the exact number included for each test is mentioned in the tables 1,2,3,4. 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 and child. The study was approved by the ethical committee of Damascus University Hospital. Mothers were informed about the study purposes and they signed in written consents. The study was performed in adherence with the guidelines of the Declaration of Helsinki.

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 minutes. Several aliquots were prepared and stored at -70°C. A volume of 50 µl of 1N acetic acid was immediately added to 500 µl of EDTA plasma and kept at -70°C for SAM and SAH assays.

25-OHD was measured by an enzyme immunoassay kit (Immuno Diagnostic Systems IDS, UK). This kit measure 25-OHD and other hydroxylated metabolites the test specificity is: 100% for 25OHD3, 75% for 25OHD2 and ≥100% for 24 25diOHD3.[36] ADMA was measured by an enzyme immunoassay kit (BioVendor, CZECH REPUBLIC). All other biomarkers (choline, betaine, DMG, MMA, cystathionine, HCY, SAM and SAH were measured as explained in our first report.[35]

The statistical analyses were performed with SPSS (version 19.0). Results are shown as mean (SD, standard deviation). Medians of continuous variables were compared between two independent groups using Mann-Whitney test. Correlations between variables were investigated using Spearman Rho test. P values below 0.05 were considered statistically significant. P values >0.05 and < 0.1 were mentioned as tendencies.

RESULTS

Table 1, shows plasma 25-OHD concentrations of children and mothers in control and CHD group according to sex of the children, maternal lifestyle and environmental factors, and maternal vitamin usage during the index pregnancy (children aged 0- 36 months). Children age in the control group (mean/ SD) (16.9 (9.4)) 47% males, children age in the CHD group (mean/ SD) (15.9 (12.3)) 42% males.

Maternal age in the control group (30.3 (5.8)), in the CHD group (27.03 (5.9)). 25-OHD in children was higher in CHD children than in controls (71.7 (61.7) vs. 45.1 (24.8)) differences were significant between plasma 25-OHD of girls in CHDs and Controls p = 0.006. Gender differences were significant in the control group p = 0.009. Plasma 25-OHD were higher in CHD children comparing to controls of families living in rural communities, having low household income, whose mothers were not consuming multivitamins during pregnancy regardless of their clothing style (veiled or unveiled) (table 1) all mentioned factors did not affect 25-OHD of CHD children (table 1).

Maternal 25-OHD levels were not different between mothers of both groups, Higher levels of plasma 25-OHD were found in control women who had consumed folic acid and other multivitamins during their pregnancy, p = 0.007, whereas other socioeconomic factors did not affect those levels in both groups with the exception of higher 25-OHD levels found in CHD mothers with lower educational status compared to control mothers, and

higher 25-OHD levels in CHD mothers resident in rural communities compared to CHD mothers in urban communities (table 1).

Table 1: 25-OHD levels in CHD and controls according to gender and different socioeconomic related status

25-OHD, nmol/L	Control n = 43	CHD n = 70	P-value*
Children (all)	45.1 (24.8)	71.7 (61.7)	0.008
Boys	51.6 (25.8)	85.4 (87.6)	0.282
N	23	29	
Girls	34.1(17.6)	64 (39.8)	0.006
N	20	41	
P$	0.009	0.179	
Urban communities	51.1 (27.9)	67.5 (37.8)	0.066
NRural communities	27	39	
N	35.5 (16.3)	78.2 (84.1)	0.005
	16	31	
p$	0.094	0.432	
Mother veiled	32.5 (12.3)	72.2 (66.3)	0.006
N	8	59	
Mother unveiled	47.9 (26.1)	67.4 (29.1)	0.046
N	35	11	
p$	0.109	0.443	
No supplements during pregnancy	48.1 (29.3)	95.5 (84.8)	0.028
N	17	41	
Multivitamins during pregnancy	42.4 (20.2)	69.8 (57.1)	0.074
N	26	29	
p$	0.759	0.333	
Household income <300$	43.4 (21.5)	85.8 (83.8)	0.089
N	8	44	
Household income > 300$	42.7 (24.8)	66.8 (33.8)	0.006
N	35	25	
P$	0.662	0.815	
Maternal education, up to high school	40.9 (18.5)	97.1 (68.4)	0.132
N	8	49	
College or higher education	43.3 (24.8)	76.8 (74.4)	0.044
N	35	21	
P$	0.957	0.818	

Table 1: 25-OHD levels in CHD and controls according to gender and different socioeconomic related status (Continued.....)

Mothers (all)	Control n = 43	CHD n = 73	P-value*
	20.2 (6.9)	21.7 (13.9)	0.508
Mothers of boys	20.4 (7.2)	21.8 (9.4)	0.536
N	21	29	
Mothers of girls	19.7 (6.7)	21.7 (16.3)	0.672
N	22	44	
p$	0.999	0.288	
Urban communities	20.1 (7.2)	20.1 (13.0)	0.216
N	24	41	
Rural communities	20.0 (6.8)	23.7 (15.0)	0.501
N	18	32	
p$	0.985	0.026	
Veiled	20.7 (8.9)	21.4 (11.5)	0.736
N	30	59	
Unveiled	19.9 (6.5)	23.6 (21.4)	0.511
N	13	14	
p$	0.880	0.337	
No supplements during pregnancy	17.4(6.3)	20.3(12.3)	0.551
N	18	43	
Multivitamins during pregnancy	24.8(6.2)	22.2(14.6)	0.049
N	23	30	
p$	0.007	0.413	
Household income <300$	23.1 (7.6)	20.2 (7.6)	0.695
N	8	48	
Household income > 300$	20.5 (7.3)	23.9 (17.2)	0.470
N	35	25	
P$	0.593	0.204	
Maternal education, up to high school	21.6 (5.7)	21.4 (14.3)	0.147
N	8	52	
College or higher education	20.5(7.8)	22.6(10.1)	0.044
N	35	21	
P$	0.957	0.818	
No previous abortion	21.9(7.3)	18.9(6.9)	0.132
N	36	46	
Previous abortion	18.8(7.2)	27.6(21.9)	0.801
N	7	17	
P$	0.286	0.201	

Data are means (SD), p values according to Mann-Whitney, $ p values between groups.

Table 2, shows results of plasma ADMA concentrations of children and mothers according to sex of the children in controls and CHDs. ADMA levels tended to be lower in children with CHD [1.92 (3.6) vs. 1.01 (0.4)] p = 0.095, this tendency was stronger in girls [2.16 (4.6) vs. 0.93 (0.32)] p= 0.066.

No gender differences in ADMA levels were found in CHDs or controls (table 2).

No differences in maternal ADMA levels of both groups (controls and CHDs), no differences in maternal ADMA either according to their children sex in both groups and inter both groups (table 2).

Table 2: 25-OHD levels in CHD and control groups according to age, gender

ADMA, µmol/L	Control n = 37	CHD n = 47	P-value*
Children (all)	1.92 (3.6)	1.01 (0.4)	0.095
Boys	1.55 (1.2)	1.12 (0.6)	0.649
N	14	21	
Girls	2.16 (4.6)	0.93 (0.32)	0.066
N	23	26	
P$	0.922	0.162	
Mothers (all)	**Control n =28**	**CHD n = 65**	**P-value***
	0.47 (0.4)	0.42 (0.3)	0.518
Mothers of boys	0.47 (0.32)	0.51 (0.4)	0.869
N	14	24	
Mothers of girls	0.47 (0.41)	0.37 (0.2)	0.984
N	14	41	
p$	0.487	0.200	

Data are means (SD), p values according to Mann-Whitney, $ p values between groups.

Figure 1, shows the correlation between plasma 25-OHD in children and their age. 25-OHD levels in CHD group tended to correlate negatively to child age (r = -0.224, p = 0.091). Nine percent of the children in the control group and 31% of those in the CHD group had 25-OHD levels higher than 75 nmol/L. 25-OHD levels in 4% of the patients exceeded 312 nmol/L.

Only 16% of CHD children and 19% of controls had levels of plasma 25-OHD less than 25 nmol/L.

Table 3 shows correlations of maternal and child 25-OHD and other metabolites in CHD group and in controls.

25-OHD in mothers and children was not correlated in CHDs (p = 0.497) nor in controls (p = 0.746), in a group of CHD children under 8 months however, P value of correlation between maternal 25-OHD and child 25-OHD was = 0.074.

Child 25-OHD in the CHD group correlated negatively to child BET/DMG (r = -0.266, p = 0.027), ADMA (r = -0.449, p = 0.004), and directly to child choline (r=0.286, p = 0.017), these levels showed a tendency in their correlation to maternal SAH (r = -0.276, p = 0.070).

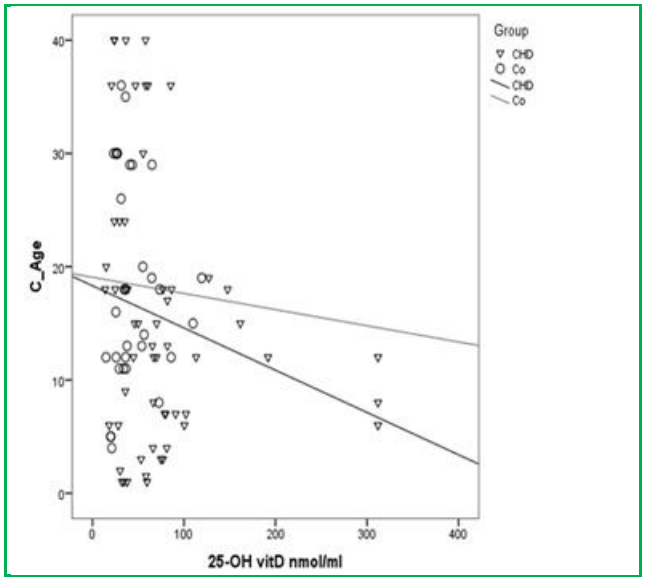


Figure 1: Correlation of plasma 25-OHD levels with children age according to groups.

All other correlations were not significant in mothers or in children of both groups (data not shown).Child 25-OHD in the control group was correlated to child hemoglobin (r = 0.426, p = 0.027), tended to correlate negatively to child choline (r=-0.279, p = 0.081), and to maternal choline (r = -0.317, p = 0.094).

Maternal 25-OHD in the control group was correlated to maternal hemoglobin (r=0.386, p = 0.027), child BET/DMG (r=0.337, p = 0.086), and child ADMA (r =-0.709, p < 0.001).

Maternal 25-OHD in the CHD group tended to correlate to maternal betaine (r = 0.210, p = 0.077).

Table 4 shows correlations of maternal and child ADMA and other metabolites in CHD group and in controls. Child plasma ADMA in CHD children tended to correlate to their maternal ADMA levels p = 0.064, correlated negatively to child BET/DMG methylation ratio (r = -0.358, p = 0.015).

Child ADMA in CHD children showed a positive tendency in its correlation to child and mother cystathionine and to maternal SAM (r =0.369, p = 0.069).

Child ADMA in the control group correlated directly to SAM/SAH methylation ratio (r=0.448, p = 0.015). and negatively to maternal 25-OHD (r=0.709, p <0.001).

Maternal ADMA correlated negatively to child MMA (r = -0.361, p = 0.008), child choline (r =-0.337, p = 0.018), child 25-OHD (r=0.449, p=0.004), and tended to correlate negatively to child folate (r =-0.328, p = 0.083).

Maternal ADMA in the control group correlated positively to maternal cystathionine (r=0.442, p=0.027) and negatively to SAM/SAH methylation ratio (r = - 0.448, p = 0.032).

Maternal ADMA correlated negatively to child choline (r = - 0.503, p = 0.012).

Table 3: Significant correlations between 25-OHD and the blood markers in mothers and children of control and CHD groups

Child or maternal biomarkers	Child 25-OHD	
	Controls	CHD patients
C-Age P N	0.121 (0.508) 32	-0.224 (0.091) 58
C-Hemoglobin P N	0.426 (0.004) 43	-0.129 (0.326) 60
C-Choline P N	-0.279 (0.081) 40	+0.286 (0.017) 70
C-BET/DMG P N	-0.014 (0.931) 40	-0.266 (0.027) 70
M-SAH P N	-0.053 (0.797) 26	-0.276 (0.070) 44
M-25-OHD P N	0.065 (0.746) 27	0.103 (0.497) 46
M-ADMA P N	-0.092 (0.675) 23	0.449 (0.004) 40
	Maternal 25-OHD	
Maternal biomarkers	Controls	CHD
M-Hemoglobin P N	0.386 (0.027) 33	-0.067 (0.620) 57
M-Betaine P N	0.080 (0.670) 31	0.210 (0.077) 72

Spearman's correlation coefficient and corresponding (P) values.

DISCUSSION

Maternal nutrition is an important causal factor related to birth defects.[37-39] Syrian women were vitamin D deficient according to their plasma 25-OHD table 1, Maternal deficiency in 25-OHD is common in the Mediterranean coast[40], however despite the low maternal 25-OHD levels in the controls as well as the CHD mothers and, despite the fact that vitamin D or any derivatives were not consumed in therapeutic doses by children of both groups controls and CHD_s, children with CHD had higher plasma 25-OHD compared to control children (71.7 vs. 45.1 nmol/L), with very high 25-OHD levels > 312 nmol/L in three CHD boys.

Several studies have linked either very low or very high serum 25-OHD levels with heart diseases.[41-42] circulating 25-OHD levels have genetic basis, some polymorphisms in genes involved in vitamin D metabolism like; DBP, VDR, CYP24 A1 were related to different circulating levels and diseases risks.[43-46] but maternal vitamin D predominates over genetic factors in determining neonatal circulating vitamin D concentrations.[47]

Table 4: Significant correlations between ADMA and the blood markers in mothers and children of control and CHD groups

Child or maternal biomarkers	Child ADMA	
	Controls	CHD patients
C-CYS P N	0.044 (0.803) 34	0.292 (0.052) 45
C-SAM/SAH P N	0.448 0.015 30	0.214 0.164 44
C-BET/DMG P N	-0.323 0.861 32	-0.358 (0.015) 46
M-Cys P N	-0.323 0.510 20	0.313 (0.081) 32
M-SAM P N	0.026 0.911 21	0.326 (0.069) 32
M-ADMA P N	-0.091 0.737 20	0.349 (0.064) 32
M-25-OHD P N	-0.709 <0.001 24	0.092 0.583 33
Maternal biomarkers	Maternal ADMA	
	Controls	CHD
M-CYS P N	0.442 0.027 20	-0.034 0.790 62
M-SAM/SAH P N	-0.448 0.032 20	-0.145 0.285 56
C-MMA P N	-0.053 0.796 26	-0.361 (0.008) 53
C-Choline P N	-0.503 0.012 24	-0.337 (0.018) 49
C-Folate P N	-0.062 0.820 20	-0.328 (0.083) 29
C- 25-OHD P N	-0.092 0.675 23	-0.449 0.004 40

Data are spearman's correlation coefficient and corresponding (P) values.

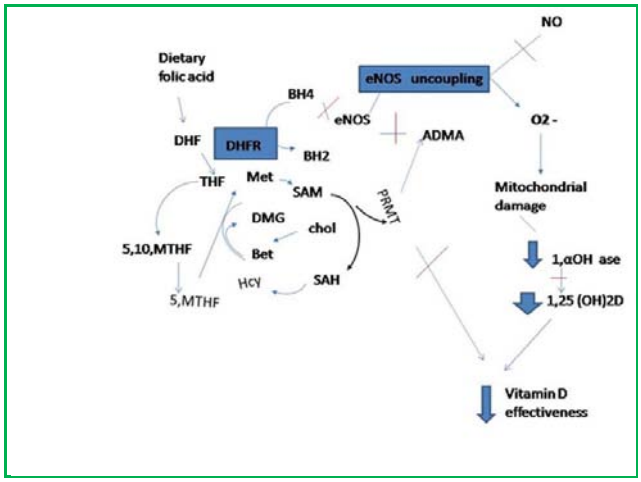
25-OHD is higher in CHD girls than in control girls (P = 0.006) while plasma ADMA in CHD girls tended to be lower (P = 0.066), 25- OHD is considered as a prohormone for the secosteroid hormone 1,25-(OH)2D, its levels reflect primarily the availability of the substrate but not

necessarily its usage, 25-OHD levels of CHD children tended to correlate with their age (p = 0.091), only in CHD children below 8 months old there was a tendency in the correlation of child 25-OHD with maternal 25-OHD (p = 0.074).

Interestingly, macrophage synthesis of 1,25(OH)2D3 requires coordinated interaction with nitric oxide (NO)[48], also, Tare et al, proved that endothelium-derived nitric oxide-evoked dilation was halved in arteries from vitamin D deficient rats.[49]

ADMA is known as a cardiovascular marker due to its role in inhibiting NOS thus preventing the vascular endothelium of NO, of importance however, in most situations where endothelial dysfunction is encountered, the expression of eNOS has been shown to be paradoxically increased rather than decreased. It appears that eNOS may become "uncoupled". In this uncoupled state, electrons normally flowing from the reductase domain of one subunit to the oxygenase domain of the other subunit are diverted to molecular oxygen rather than to L-Arg; in these conditions, superoxide rather than NO is produced. Superoxide produced from the uncoupled NOS leads to a state of oxidative stress.[50] The most important cause for this NOS uncoupling state is the loss of the critical NOS cofactor biopterin BH4 either by oxidation or by decreased activity of the recycling enzyme dihydrofolate reductase (DHFR).[51] ADMA levels in CHD children tended to correlate to maternal ADMA levels (p = 0.064), and maternal ADMA is correlated negatively to child 25-OHD (n = 40, p = 0.004), child choline (n = 49 , p = 0.012), child methylmalonic acid (n = 49, p = 0.008), and tended to correlate negatively also to their folate levels (n = 29 , p = 0.083) table 3, these correlation in CHD patients demonstrated the intersection between folate cycle and ADMA levels hence NO production (Figure 2).

ADMA mean plasma levels tended to be lower rather than higher in CHD children (1.01(0.4) vs. 1.92(3.6)). This decrease in ADMA as well as the one carbon metabolism abnormal biomarkers might contribute to NOS uncoupling. In view of the important role of nitric oxide and its generating enzyme, in generalized vascular function[52], it is probable that they will also influence endothelial cell mitochondrial cytochrome 1α-OHase (one alpha hydroxylase) or B27 (responsible for the second hydroxylation of 25-OHD to the active form 1,25-(OH)2D)[53] (figure 2). An increased production of ROS by uncoupled eNOS contributes markedly to the pathophysiology in the coronary and peripheral circulation and has important prognostic implications for subsequent cardiovascular events. A reduced convergence of 25- OHD to its active form could also be due to reduced mitochondrial cytochrome B 27 activity caused by oxidative stress.[54] This oxidative/ NO damage either caused by nutritional deficiencies and / or genetic susceptibilities will be transferred to the foetus as oocytes have up to 100,000 mitochondria[55], methylation was reported as a direct mechanism in regulating vitamin D metabolism since 24-hydroxylase (CYP24A1) promoter methylation plays an important unique role in vitamin D catabolism in the placenta[15], also PRMT4 as mentioned earlier is vital to the response to vitamin D signal[21]. Protein methylation is more prone to inhibition by SAH than DNA methylation.[56] In our results, 25-OHD in CHD children tended to correlate negatively with their maternal SAH (p = 0.070) furthermore, 25-OHD levels in CHD children correlated negatively to their BET/DMG methylation ratio (p = 0.027), this ratio was reported lower in CHD children than controls in our earlier report.[35] These results indicate that The presence of 25-OHD in CHD children is correlated to these patients methylation of DNA and Proteins status, in either possible cases, low convergence to 1,25-(OH)2D (aberrant methyl cycle causing aberrant No/ oxidative status and low 1α-OHase), or the absence to the response to active vitamin D signal and thus its action (aberrant methyl cycle causing low activity of PRMT 4 needed for vitamin D response) (Figure 2), an increase in 25-OHD as a prohormone could be encountered.



1α OH ase:1 alpha hydroxylase, 5,10 MTHF: 5, 10 methylene tetrahydrofolate, 5 MTHF: 5 methylene tetrahydrofolate., ADMA: Asymmetric dimethyl arginine, BH2: dihydrbiopterin, BH4: tetrahydrobiopterine, DMG: dimethylglycine, DHF: Dihydrofolate, HCY: homocysteine, MMA: methylmalonic acid, PRMT: protein methyl transferase, SAM: S-adenosylmethionine, SAH: S-adenosylhomocysteine, THF: tetrahydrofolate.

Figure 2: shows the possible interaction between one carbon metabolism, Protein methylathion and the vitamin D metabolism.

The current study has few limitations. First, the reason of elevated 25-OHD in CHD children is not known and a causality link to CHD cannot be assumed. Some differences between the study groups showed only tendency suggesting that a larger sample size would be needed to draw a final conclusion; other important markers for vitamin D status should be considered in future studies and peripheral impaired 25-OHD uptake should be investigated.

CONCLUSION

To our best of knowledge we are the first to demonstrate high 25-OHD levels in this group of patients and its relation with one carbon metabolism biomarkers in CHD Children as well as their mothers. This finding may elucidate new aberrant metabolic pathway that may cause adverse birth outcome, and emphasizes the role of epigenetic in vitamin D metabolism.

The status and the role of vitamin D and ADMA in the pathogenesis of CHD deserve further investigation.

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