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PRACA ORYGINALNA
ORIGINAL PAPER

Homocysteine remethylation pathway in neonates with congenital heart disease and neural tube defects

Szlak remetylacji homocysteiny u noworodków z wrodzonymi wadami serca i wadami cewy nerwowej

Piotr Surmiak[1], Małgorzata Baumert[1], Zofia Walencka[1], Magdalena Paprotny[1], Andrzej Witek[2]

[1]Department of Neonatology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland
[2]Department of Gynaecology and Obstetrics, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

ABSTRACT

INTRODUCTION: The etiology of congenital heart defects (CHD) and neural tube defects (NTD) remain unknown, however, the relation between homocysteine and folate levels and congenital anomalies were found. With this perspective in mind, the aim of the study was to investigate serum biomarkers of the homocysteine metabolism pathway in neonates with CHD, newborns with NTD and their mothers.
MATERIALS AND METHODS: Twenty-nine pairs of mothers and their neonates with CHD as well as 18 pairs of mothers and neonates with NTD were enrolled in the study. The control group consisted of 54 pairs of mothers and their healthy neonates. To estimate the total homocysteine, serum folic acid and vitamin B_{12} levels in plasma, mothers' venous blood samples and umbilical cord blood were taken in the all groups.
RESULTS: There were significantly higher tHcy levels in the newborns with CHD compared to their mothers. The total homocysteine levels in the CHD neonates were noticeably different compared to the neonates with NTD and to the controls. The vitamin B_{12} levels were similar in all the investigated neonates. Significantly lower umbilical folic acid levels in the NTD and CHD groups as compared to the controls were noticed.
CONCLUSIONS: The observed differences in concentrations of homocysteine, folic acid and cobalamin between neonates with congenital heart and neural tube defects suggest the influence of various agents disturbing the homocysteine metabolic pathways in those children.

KEY WORDS
homocysteine, neural tube defect, congenital heart disease, folate, cobalamin

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Address for correspondence: Dr n. med. Piotr Surmiak, Department of Neonatology, School of Medicine in Katowice, Medical University of Silesia, Katowice, ul. Medyków 14, 40-752 Katowice, Poland, tel. + 48 509 792 558, e-mail: piotrek.surmiak@gmail.com

STRESZCZENIE

WSTĘP: Etiologia wrodzonych wad serca (WWS) oraz wad cewy nerwowej (WCN) jest niewyjaśniona, wykazano jednak związek między stężeniem homocysteiny oraz kwasu foliowego a występowaniem wrodzonych anomalii.
Celem pracy była ocena stężenia biochemicznych markerów szlaku metabolizmu homocysteiny u noworodków z WWS oraz WCN i ich matek.
MATERIAŁ I METODY: Badaniem objęto 47 par – matka i jej noworodek urodzony z WWS (n = 29) i WCN (n = 18). Grupę kontrolną (GK) stanowiły 54 pary matek i ich zdrowe dzieci. Próbki krwi matki i krwi pępowinowej pobierano w celu oznaczenia stężenia tHcy, kwasu foliowego i witaminy B_{12}.
WYNIKI: Stężenie tHcy u noworodków z WWS było znamiennie wyższe niż u ich matek oraz u dzieci z WCN. Stężenie tHcy u noworodków z WCN było znamiennie wyższe niż w grupie kontrolnej. Stężenia witaminy B_{12} były podobne u wszystkich badanych noworodków w porównaniu z wartościami stwierdzonymi u ich matek. U noworodków z WCN i WWS stężenie kwasu foliowego w krwi pępowinowej było znamiennie niższe niż u dzieci z grupy kontrolnej.
WNIOSKI: Zaobserwowane różnice w stężeniu homocysteiny i kwasu foliowego między noworodkami z wrodzonymi wadami serca i wadami cewy nerwowej sugerują obecność dodatkowych czynników zaburzających szlaki metaboliczne homocysteiny u tych dzieci.

SŁOWA KLUCZOWE
homocysteina, wrodzona wada serca, kwas foliowy, kobalamina, wada cewy nerwowej

INTRODUCTION

The most common problem of contemporary perinatology is the high number of inborn developmental defects, of which heart and neural tube defects are two of the most common anomalies in the developmental period [1,2]. The etiology of many inborn developmental defects remains unknown. According to current knowledge, more than 80% of defects with recognized etiology are stimulated by genetic and environmental factors [2]. Chief among the aforementioned influences is a deficiency of essential microelements and vitamins in the preconception period, such as B-vitamins (i.e. folic acid and cobalamin). There are reports suggesting that folates play a key role in the methylation reactions of many biogenic compounds, including DNA [3]. This reaction is considered to be the main mechanism responsible for the expression of genes in multipotential cell differentiation at the stage of early embryogenesis. Low concentrations of folic acid and vitamin B_{12} cause homocysteine (tHcy) accumulation in cells as a result of defective utilization [4]. Hyperhomocysteinemia (HHcy) is one of the main factors which cause inborn defects, including congenital heart defects (CHD), neural tube defects (NTD) and the development of many other civilization diseases [4,5,6].
The aim of the study was to evaluate the differences in the concentration of homocysteine, folic acid and vitamin B_{12} in mothers' serum and the umbilical cord blood of newborns with congenital heart and neural tube defects.

MATERIALS AND METHODS

A prospective, observational study was conducted in the Department of Neonatology at the Medical University of Silesia in Katowice between January 2011 and January 2014.
From among 1,271 childbirths in our Unit at the time of the study, after approval by the Human Ethics Committee of the Medical University of Silesia and informed parental consent, we enrolled 29 neonates with prenatally diagnosed critical congenital heart defects (CHD) and 18 neonates with prenatally diagnosed neural tube defects (NTD), as the study groups. The control group comprised 54 pairs of healthy, full-term newborns and mothers residing in the unit during the study.
All the mothers from the study group underwent prenatal examinations at 20–23 weeks of gestation, which is a standard protocol for all pregnant women in our hospital.
The group of neonates with congenital heart defects was diagnosed as follows: hypoplastic left heart syndrome (n = 11; 38%), common arterial trunk (n = 6; 20%), coarctation of the aorta (n = 7; 24%), double outlet right ventricle (n = 3; 10%), and hypoplasia of pulmonary arteries (n = 2; 7%).

The newborns with neural tube defects were diagnosed with: spina bifida (n = 7; 39%), cerebral hernia (n = 6; 33%), meningocoele (n = 3; 16%) and holoprosencephaly (n = 2; 11%). All the defects were reported to the Polish Registry of Congenital Malformations (PRCM).

The exclusion criteria comprised: neonates with chromosomal aberrations, complex congenital malformations, newborns from multiple pregnancies; neonates with evidence of congenital infections, as well as those that were born to mothers with clinical chorioamnionitis. We also excluded mothers who administered medications during pregnancy, which could possibly affect homocysteine and vitamin B metabolism (i.e. folate antagonists, antiepileptic drugs, oral contraceptives, barbiturates, Levodopa) within the period of six months before conception, as well as pregnant women suffering from hypertension, thromboembolic diseases, kidney and heart defects.

The women from both the case and control groups were Caucasian, under 35 years of age, with adequate renal function (glomerular filtration rate, GFR based on serum creatinine level and ranged between 80 and 120 ml/min/1.73m^2).

The neonates from the control group underwent a cranial ultrasound and echocardiographic examination on the third day after delivery.

Laboratory tests

During childbirth, 5 milliliters of blood was collected from the mother's ulnar vein and umbilical artery from the placental side, as a standard protocol of our hospital.

All the blood samples were collected in EDTA-containing tubes, centrifuged for 10 minutes (2500 rotations/min) and stored at -70°C until full analysis had been performed. This procedure was recommended by the ABBOTT company which produces the test sets used in our examination. The following was assessed: folic acid, vitamin B_{12} and total homocysteine (tHcy) concentrations. Folic acid and vitamin B_{12} in serum were determined with the aid of the micro-particle enzyme immunoassay (MEIA), using ABBOTT reagent sets in an immunochemical analyzer (AxSYM). The total homocysteine concentration in plasma was determined by an immunochemical method with the fluorescence polarization immunoassay (FPIA) using an IMx analyzer and special ABBOTT sets.

Statistical analysis

The database created made in Excel, and the results were analyzed statistically with the certified program STATISTICA 10 (StatSoft Polska Inc.). As the distribution of the data was skewed, they were analyzed by the Shapiro-Wilk test, and the results are expressed as median and interquartile ranges or as percentiles of the total. The baseline characteristics between the case and control groups were compared using the Chi-square or Fisher's exact tests.

The biomarker concentrations of the case and control groups were compared using the Mann-Whitney U test and Kruskal-Wallis test. The association between the variables was measured by Spearman's rank correlation test. For all the statistical procedures, a *p*-value < 0.05 was considered to be significant.

RESULTS

The neonates from the study groups were comparable to the control with respect to demographic-perinatal characteristics, except for the Apgar score at the fifth minute after delivery, and it was significantly lower in both study groups ($p = 0.04$) (Tab. I).

In our study, the tHcy concentration was significantly higher in the newborns with CHD in comparison to their mothers, neonates with NTD and to the controls. Additionally, the tHcy concentration in the newborns with NTD was significantly higher than in the control group. We observed no differences between the tHcy levels in the neonates with NTD and their mothers. No differences were shown in the tHcy levels observed among the mothers of any of the investigated newborns. All the results are presented in Table II and Figure 1.

In our study, we did not notice any relevant differences in the vitamin B_{12} concentrations among the children from any of the groups. There was no noticeable differences in the concentration of folic acid in the mothers from either of the study groups. The concentrations of vitamin B_{12} were similar in all the observed mothers (Tab. II and Fig. 2).

In the neonates with NTD and CHD, it was recognized that there was a significantly lower umbilical folic acid level in comparison to the control group.

The neonates born with NTD exhibited a significantly lower folic acid concentration in comparison to their mothers. The mothers' folic acid serum levels did not show any difference in any of the groups (Tab. II and Fig. 3).

Additionally, it was determined that there was a negative correlation between the concentrations of tHcy and folic acid ($r = -0.64$; $p = 0.002$), as well as vitamin B_{12} ($r = -0.31$; $p = 0.04$) in the CHD mothers group while a positive correlation between folic acid and vitamin B_{12} ($r = 0.59$; $p = 0.001$) was noted in all the investigated mothers. We also observed a negative correlation between the folic acid levels in the mothers from the study groups and that of homocysteine in their children ($r = -0.63$; $p = 0.03$).

Table I. Perinatal characteristics of study neonates (neural tube defects, NTD and congenital heart defects, CHD) and controls
Tabela I. Charakterystyka okołoporodowa badanych noworodków (wady cewy nerwowej oraz wrodzone wady serca) oraz z grupy kontrolnej

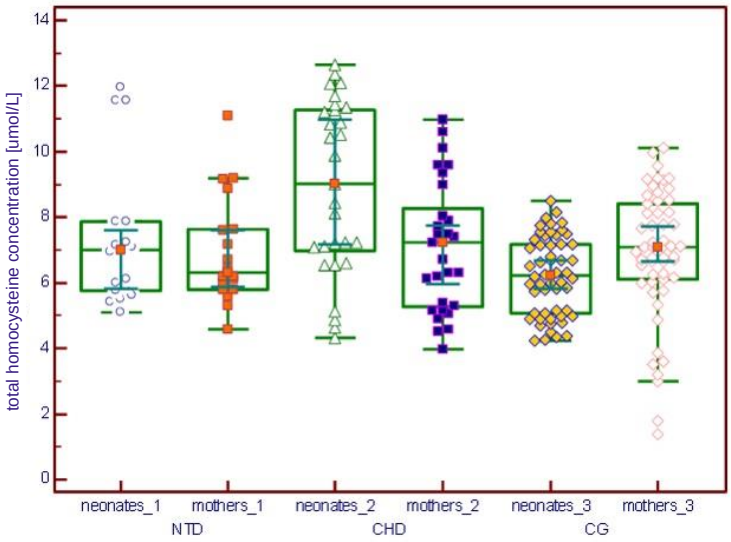
	Neural Tube Defects (n = 18)	Congenital Heart Defects (n = 29)	Control Group (n = 54)
Mothers			
age (years)	28 [25–33]	30 [25–34]	29 [26–32]
dietary supplementation of folic acid during pregnancy (n; %)	16; 88.9%	23; 93.1	49; 90.7
Newborns			
gestational age (wks)	38 [37–39]	39 [38–40]	39 [39–40]
gender female [n; %]	10; 55.6%	14; 48.3%	32; 59.3%
birth weight (g)	2890 [2670–3050]	2750 [2450–3160]	2940 [2520–3110]
head circumference (cm)	34.0 [33.5–34.5]	34.5 [34–35.5]	35.0 [34–36]
General condition after birth			
Apgar 1st min (pts)	7 [7–8]	8 [7–8]	9 [8–10]
Apgar 5th min (pts)	9 [8–10]	9 [8–10]	10 [9–10]

Results shown as medians and 95% confidence intervals or percentile.

Table II. Total homocysteine (tHcy), folate and cobalamin levels in umbilical cord blood and mothers' venous blood samples in all investigated groups
Tabela II. Stężenie całkowitej homocysteiny, folianów oraz kobalaminy w krwi pępowinowej oraz w matczynej krwi żylnej we wszystkich analizowanych grupach

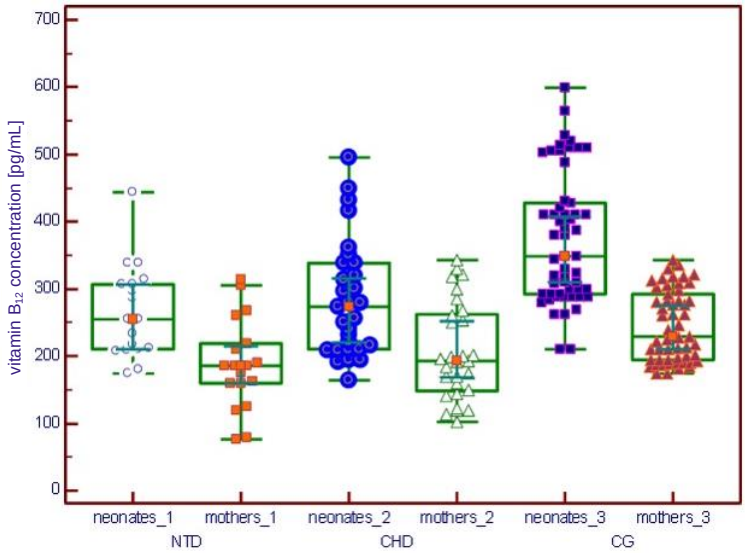
Variable	Neural Tube Defects (n = 18)	Congenital Heart Defects (n = 29)	Controls (n = 59)	*p* value
Umbilical cord blood				
tHcy level [µmol/l]	8.2 [2.4]	9.0 [2.7]	6.2 [1.2]	0.01
folate level [ng/ml]	7.2 [4.5]	12.9 [4.7]	15.2 [2.7]	< 0.001
cobalamin level [pg/ml]	279.3 [77.2]	290.0 [88.6]	327.3 [96.5]	0.3
Mothers				
tHcy level [µmol/l]	7.2 [1.6]	7.3 [1.9]	6.9 [2.0]	0.5
folate level [ng/ml]	10.3 [3.3]	10.1 [3.0]	13.0 [2.1]	0.9
cobalamin level [pg/ml]	189.9 [71.9]	211.9 [73.6]	244.8 [52.2]	0.1

Results presented as means and standard deviations; *p* value from Kruskal-Wallis test.



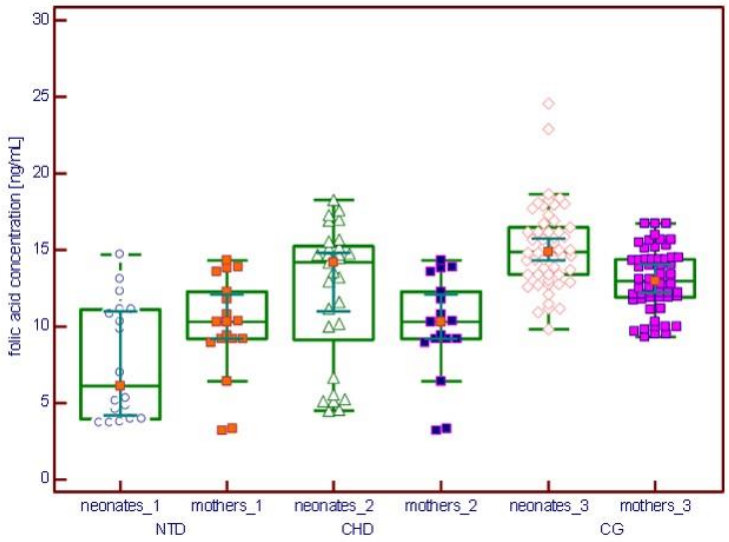
Results presented as medians and 95% confidence intervals, as well as minimum and maximum values.

Fig. 1. Maternal and umbilical cord blood total homocysteine concentrations in all investigated groups: neural tube defects (NTD), congenital heart defects (CHD) and controls (CG).
Ryc. 1. Stężenie całkowitej homocysteiny we wszystkich analizowanych grupach w krwi matek i w krwi pępowinowej: wady cewy nerwowej (WCN), wrodzone wady serca (WWS) oraz kontrola (GK).



Results presented as medians and 95% confidence intervals, as well as minimum and maximum values.

Fig. 2. Maternal and umbilical cord blood vitamin B_{12} concentrations in all investigated groups: neural tube defects (NTD), congenital heart defects (CHD) and controls (CG).
Ryc. 2. Stężenie witaminy B_{12} we wszystkich analizowanych grupach w krwi matek i w krwi pępowinowej: wady cewy nerwowej (WCN), wrodzone wady serca (WWS) oraz kontrola (GK).



Results presented as medians and 95% confidence intervals, as well as minimum and maximum values.

Fig. 3. Maternal and umbilical cord blood folic acid concentrations in all investigated groups: neural tube defects (NTD), congenital heart defects (CHD) and controls.
Ryc. 3. Stężenie kwasu foliowego we wszystkich analizowanych grupach w krwi matek i w krwi pępowinowej: wady cewy nerwowej (WCN), wrodzone wady serca (WWS) oraz kontrola (GK).

DISCUSSION

In Poland, the frequency of neural tube defects ranges from 6.7 to 8.6 per 10 000 live births, and that of heart defects 70 per 10 000 live births [1,2]. The percentage of the above-mentioned defects is higher in Poland than in most European countries, due to the fact that in Poland there are fewer cases of pregnancy terminations after the diagnosis of developmental anomalies during prenatal examinations. According to the Papiernik et al. study, Poland is among the countries with the lowest frequency of abortions due to developmental defects [7].

Our report is the first study examining the relationships between concentrations involved in one-carbon metabolism (homocysteine, folate and vitamin B_{12}) in mothers and their offspring with congenital malformations (CHD and NTD). It has been well documented that HHcy is associated with the occurrence of congenital defects, including neural tube closure failure and conotruncal septal anomalies in the heart [8,9,10, 11]. HHcy is considered to be one of the independent etiological agents stimulating inborn, structural defects [7,10,11]. The impact of HHcy on embryonic precursor cells leads to defective fetal development, which results in impaired closure of the neural tube and abnormal development of the aorticopulmonary septum [6]. The teratogenic effect of Hcy on the abovementioned cells has been proved by animal testing [12,13,14].

Some authors have suggested that developmental abnormalities of the neural tube and conotruncal septal anomalies of the heart are associated with low maternal folate as well as with hyperhomocysteinemia [15,16]. However, in our study, we did not unveil any differences in folic acid and homocysteine levels in the mothers of the NTD, CHD neonates and the controls (all the mothers received daily multivitamin supplementation containing the recommended dose of folic acid).

We postulated that the mothers' folic acid and homocysteine levels had no direct influence on the development of congenital malformations. Thus, we suggested that HHcy in neonates with NTD and CHD is connected with metabolism disturbances within the fetus.

According to our hypothesis, a defective remethylation pathway is responsible for heart defects, although there is a common origin of precursor cells of the heart and neural tube. Hence, the pathomechanism of the formation of these defects is probably different and it may concern Hcy utilization pathways. This is similarly presented by Zhao et al., who found in children with CHD a gene mutation coding for an enzyme which plays an important role in the process of homocysteine remethylation [17].

It is probable that in fetuses with NTD excess Hcy is metabolized by remethylation as we discovered a decreased concentration of folic acid in children with NTD compared to those with CHD. This theory is in accordance with Saxena et al., who observed in offspring with NTD a gene mutation for the key enzyme of the transsulfuration pathway [18]. Nevertheless, the study results are not consistent. For example, Richter et al., presented in their article that the

main cause of NTD is a defective remethylation pathway [19].

According to Solanky et al., the remethylation of Hcy to methionine using methyl donation from folate is the prevalent pathway in the human placenta, indicating a marked reliance on folate availability [20]. Consequently, vitamin B deficiency in mothers whose offspring have congenital malformations additionally cause a disturbance in the main pathway of Hcy remethylation in the placenta and results in an increased transfer of Hcy from maternal to fetal circulation.

Therefore, confirmation by future prospective multicentre cohorts is needed.

CONCLUSIONS

The observed differences in the concentrations of homocysteine, folic acid and cobalamin between neonates with congenital heart and neural tube defects suggest the influence of various agents disturbing the homocysteine metabolic pathways in those children.

Author's contribution

Study design – P. Surmiak, M. Paprotny
Data collection – M. Paprotny, Z. Walencka
Data interpretation – P. Surmiak, M. Baumert
Statistical analysis – P. Surmiak, M. Paprotny, M. Baumert
Manuscript preparation – P. Surmiak, M. Baumert, A. Witek
Literature research – P. Surmiak, Z. Walencka

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