P R A C E   O R Y G I N A L N E

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Plasma homocysteine concentrations in mothers and term and preterm newborns

Stężenie homocysteiny we krwi noworodków urodzonych o czasie i przedwcześnie oraz ich matek

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Abstract

Aim: *To assess the correlation between homocysteine concentrations and gestational age, gender, Apgar score, complications in pregnancy, delivery modalities and levels of vitamin B12 and foliate.*

Material and methods: *Concentration of homocysteine, vitamins-B12, foliate were measured in cord blood and mother blood. There were 40 full term babies and 38 preterm babies and their mothers.*

Result: *The homocysteine concentration in newborns correlated with homocysteine level in mothers. There was no difference in homocysteine level regardless of newborn's gender.*

There was no correlation in the homocysteine concentration of mother's blood and cord blood with the levels of vitamin B12 and foliate.

In full term newborns a significant increase in homocysteine levels in comparison with premature babies was observed (7.2±1.4μmol/l vs. 6.4±1.3μmol/l; p=0.01). Additionally, negative correlation between the mothers' age and homocysteine concentration (r=-0.23; p=0.04) and positive correlation between homocysteine concentration in cord plasma and gestation age (r=0.28; p=0.01) were found.

Conclusion: *Homocysteine concentration depends on gestational age, Apgar score and mother's age. There is no correlation between homocysteine level and hypertension during pregnancy, type of delivery, levels of vitamin B12 and foliate. Determination of homocysteine level is therefore of no significant importance in newborns pathophysiology.*

Key words: **homocysteine** / **term infants** / **premature infants** /

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Streszczenie

Cel pracy: *Wykazanie, czy istnieje korelacja pomiędzy stężeniem homocysteiny a wiekiem płodowym, płcią, stanem ogólnym noworodków ocenianym w skali Apgar, problemami w ciąży, rodzajem porodu i stężeniem witaminy B12 i kwasu foliowego.*

Materiał i metody: *Badano stężenie homocysteiny, witaminy B12, kwasu foliowego w surowicy krwi pępowinowej i w krwi matek. Noworodków donoszonych było 40, a wcześniaków 38.*

Wyniki: *Stężenie homocysteiny u dziecka korelowało ze stężeniem homocysteiny u matki. Nie stwierdzono różnic istotnych statystycznie w stężeniu homocysteiny u noworodków w zależności od płci dziecka. Nie wykazano korelacji stężenia homocysteiny zarówno u matki jak i w krwi pępowinowej ze stężeniem witaminy B12 i kwasu foliowego. Wykazano istotnie wyższe stężenia homocysteiny u noworodków urodzonych w terminie porodu w porównaniu z noworodkami urodzonymi przedwcześnie (7,2±1,4μmol/l vs. 6,4±1,3μmol/l (p=0,01). Stwierdzono ujemną korelację pomiędzy wiekiem matki i stężeniem homocysteiny (r=-0,23; p=0,04) i dodatnią korelację pomiędzy stężeniem homocysteiny w krwi pępowinowej i wiekiem płodowym (r=0,28; p=0,01).*

Wnioski: *Stężenie homocysteiny w krwi pępowinowej zależy od wieku płodowego, stanu po urodzeniu wg Apgar i wieku matki. Stężenie homocysteiny w krwi pępowinowej nie koreluje z nadciśnieniem w czasie ciąży u matki, rodzajem porodu, ani też nie zależy od stężenia witaminy B12 i kwasu foliowego. Oznaczanie stężenia homocysteiny w krwi pępowinowej nie stanowi użytecznego wskaźnika w patofizjologii noworodka.*

Słowa kluczowe: **homocysteina** / **noworodek donoszony** / **wcześniak** /

Introduction

Homocysteine is derived from the essential amino acid methionine and its metabolism depends on the B vitamins-cobalamin (vitamin B12), pyridoxine (vitamin B6), and folic acid [1]. Previous studies have shown that hyperhomocysteinemia is an independent risk factor of premature disease, stroke, coronary artery disease and venous thromboembolism, as well as neuropsychiatric diseases [2].

The mechanism of the damaging effect of homocysteine is very complex and not completely explained. It has been proven that homocysteine particles directly damage the endothelium in vessels; they intensify the oxidation of low density lipoproteins (LDL) and they increase the aggregation of platelets [3]. They also stimulate the proliferation of smooth muscles in blood vessels and intensify oxidation stress. It has recently been proven that homocysteine can cause damage to collagen structures in blood vessels and destroy DNA cells, which leads directly to dysfunction or even the death of the endothelium [4, 5].

The aim of the study was to assess the correlation between homocysteine concentrations and gestational age, gender, Apgar score, complications in pregnancy, delivery modalities and levels of Vitamin B12 and foliate.

Material and methods

The protocol was approved by ethics committee at Silesian Medical University, Poland.

This prospective study included 78 pregnant women and their fetuses that were monitored from January, 2008 until December, 2008. There were 38 preterm babies and 40 full term babies.

Maternal blood samples were collected via venipuncture at the time of admission for delivery. Umbilical cord blood was collected by direct venipuncture of the umbilical vein after the delivery of the fetus before the passage of placenta. All blood samples were collected in 5ml EDTA tubes.

Samples were centrifuged at 3000 r.p.m for 10 minutes. Plasma was extracted, frozen and maintained at -70°.

Homocysteine concentrations of maternal and umbilical plasma were measured using MEIA methods (Abbott, USA) with the laboratory reference values 4.45-12.42μmol/l and whose production norm is 9.68 μmol/l.

The concentrations of vitamin B12 and folic acid were measured using MEIA methods (Abbott, USA). The laboratory reference value for vitamin B12 concentration was 160-800 pg/ml and for foliate 5.3-14.4ng/ml.

The study protocol was approved by the Ethics Committee at the Medical University of Silesia and all participants gave their written informed consent. During pregnancy all women had been regularly prescribed a daily dose of vitamins containing 0.8 mg of folic acid, 4 μg of vitamin B12 and 2.6mg of vitamin B6.

Statistical methods

Quantitative variables are expressed as the arithmetic means with standard deviation (normally distributed variables) or as medians with interquartile range (those not normally distributed). Categorical variables are presented as crude values.

Data were tested for normality with the Shapiro-Wilk W test. The analysis was performed in two stages. The first stage of simple bivariate analysis included between-group comparisons and the assessment of the relationship between variables.

Associations between quantitative variables were assessed on the basis of Pearson's or Spearman rank correlation coefficients. Between-group differences were tested using the Student t-test or the Mann-Whitney U test as appropriate.

For qualitative variables chi-squared or Fisher exact tests were used. P<0.05 was considered statistically significant.

Results

Characteristics of examined patients with the distinction between newborns' gender, is presented in Table I.

Boys had a statistically higher birth weight in comparison with girls (p=0.003).

Table I. Characteristics of the examined groups in relation to gender of newborns.

Parameter		Baby-girl	Baby-boy	All	'p'
Gender (n)		41	37	78	–
Gestational age (wk)	<37	19	19	38	0.8
	≥37	22	18	40	
Mother's age (yr)		30.4 ±5.6	29.6 ±4.2	30.0 ±5.0	0.3
Mode of delivery	vaginal	16	14	30	0.9
	CS	25	23	48	
Apgar score (pts) in 1st min	0-4	6	3	9	0.6
	5-7	19	17	36	
	8-10	16	17	33	
Birth weight (g)		2449.0 ±887.8	2918.1 ±724.4	2671.5 ±842.8	0.03
Disorder during pregnancy	HT	7	8	15	0.9

CS – cesarean section, HT – hypertension

Table II. Homocysteine, vitamin B12 and foliate levels in maternal plasma and umbilical cord plasma in relation to gender of newborns.

Parameter	Baby-girl	Baby-boy	All	'p'
Umbilical cord plasma homocysteine levels [μmol/l]	7.0 ±1.6	6.6 ±1.2	6.8 ±1.4	0.2
Mother's plasma homocysteine levels [μmol/l]	8.5 ±1.8	8.0 ±1.4	8.3 ±1.6	0.2
Umbilical cord plasma vitamin B12 levels [pmol/l]	309.8 ±88.0	328.6 ±79.8	318.7 ±84.2	0.4
Mother's plasma vitamin B12 levels [pmol/l]	222.2 ±54.9	225.4 ±45.5	223.7 ±50.4	0.4
Umbilical cord plasma foliate [nmol/l]	12.9 ±2.5	13.3 ±2.5	13.1 ±2.5	0.5
Mother's plasma foliate levels [nmol/l]	12.6 ±2.0	12.7 ±1.7	12.7 ±1.9	0.7

Table III. Factors determining homocysteine concentrations in newborns and mothers.

Parameter		Umbilical cord homocysteine level [μmol/l]	'p'	Mathernal homocysteine level [μmol/l]	'p'
Gender	girls	7.0 ±1.6	0.2	8.5 ±1.8	0.2
	boys	6.6 ±1.2		8.0 ±1.4	
Gestational age (wk)	<37	6.4 ±1.3	0.01	7.9 ±1.4	0.06
	≥37	7.2 ±1.4		8.6 ±1.8	
Mode of delivery	vaginal	7.6 ±1.4	<0.001	9.0 ±1.7	<0.001
	Cs	6.4 ±1.3		7.8 ±1.4	
Cesarean section	<37	6,3±1,3	>0.1	7,9±1,4	>0.1
(Cs)	≥37	6,2±1,1		7,5±1,5	
Apgar score in 1st min (pts)	0-4	6.5 ±1.0	0.04	8.0 ±1.3	0.2
	5-7	6.5 ±1.6		8.0 ±1.6	
	8-10	7.3 ±1.2		8.7 ±1.8	
Disorder during pregnancy	HT	6.7 ±1.1	0.3	8.2 ±1.2	0.1

CS – cesarean section, HT – hypertension

Table IV. Results of analysis of linear correlation.

	Maternal homocysteine	Umbilical cord homocysteine	Umbilical cord vitamin B12	Umbilical cord foliate
Maternal homocysteine	-	r=0.86; p<0.01	-	-
Maternal vitamin B12	r=0.14; p=0.2	r=0.19; p=0.1	r=0.44; p<0.01	-
Umbilical vitamin B12	r=0.04; p=0.7	r=-0.07; p=0.5	-	-
Maternal foliate	r=-0.04; p=0.1	r=-0.14; p=0.2	-	r=0.58; p<0.01
Umbilical cord foliate	r=0.06; p=0.6	r=-0.08; p=0.5	-	-

There were no statistically significant differences between boys and girls regarding the gestational age, number of pregnancy, single or multiple pregnancy, the state of newborns according to Apgar score in the 1st minute after delivery were found. Table II presents the concentration of homocysteine, B12 vitamin, foliate in mother's blood and their newborns blood.

The concentration of homocysteine in mothers' blood correlated with the value in umbilical cord blood (r=0.86 p<0.0001). (Figure 1).

This relationship was observed in term newborns as well as in preterm newborns. (r=0.88, p<0.001 and r=0.82, p<0.001, respectively).

There were no statistically significant differences in the homocysteine concentration in newborns with the distinction between the newborns' gender. (Table III).

There was a significantly higher homocysteine concentration in term newborns in comparison with preterm newborns (7.2±1.4 µmol/lvs. 6.4± 1.3µmol/l; p=0.01) but those differences were not found in homocysteine concentrations in mothers of term and preterm newborns. (Table III).

Mothers and babies born by natural labour had a significantly higher homocysteine concentration in comparison with mothers and newborns delivered by elective Caesarean section (p<0.001). (Table III).

It was observed that newborns in good condition had a significantly higher homocysteine concentration in comparison with newborns delivered in a moderate or critical condition (p=0.04). (Table III).

Hypertension during pregnancy did not affect the concentration of homocysteine in either the mother's blood or umbilical cord blood. (Table III).

The concentration of vitamin B12 and foliate levels in mother's blood correlated with the concentrations in umbilical cord blood (r=0.44; p<0.01- Vit B12; r= 0.58; p<0.01- foliate) in all examined patients. (Table IV).

There was no correlation in the homocysteine concentration of mother's blood and umbilical cord blood with the levels of vitamin B12 and foliate. (Table IV).

We observed a positive correlation between fetal age and the homocysteine concentration in umbilical cord blood (r=0.28; p=0.01). (Figure 2), and negative correlation between the mother's age and the homocysteine level in umbilical cord blood (r=-0.23; p=0.04). (Figure 3).

Discussion

The concentration of homocysteine in pregnant women decreases in the first trimester of gestation in comparison with women who are not pregnant. Then it increases and reaches the highest level in the third trimester of gestation [6].

The pregnant women, who participated in our observations, had a mean concentration of homocysteine 8.3±1.6µmol/l. Those levels are the accepted norms. The concentration of homocysteine in all examined newborns showed levels within the accepted norms for adults and was 6.8±1.4µmol/l. A significant relation between the concentration of homocysteine in the cord blood and the gestation age was observed by Hongsprabhas [7].

Also in our study the concentration of homocysteine was higher in term newborns (7.2±1.4µmol) than in preterm newborns (6.4±1.3µmol) (p=0.01**)**. However those differences in homocysteine levels could arise from mode of delivery because term newborns were delivered both by Cesarean section and natural, but preterm newborns only by Cesarean section. It is confirmed by our further observations in which we showed that there were no differences in homocysteine concentration between term and preterm infants born by Cesarean section. We also observed no significant differences in concentrations of homocysteine in the mother's blood in both groups. Concentration of homocysteine in mothers who delivered at term was higher that in mothers who had preterm deliveries. Presumably the increasing concentration of homocysteine affects the spontaneous gestational uterus contractions [8]. Our study shows that newborns born by natural labour had a significantly higher concentration of homocysteine than the newborns born by elective Caesarean section. There is a possibility that this is connected with the higher concentration of cortisol, which increases due to the stress labor. Umbilical cord plasma homocysteine levels correlate with the corresponding maternal plasma levels. The results suggested fetal and neonatal metabolic adaptation to peri-partal events. It is known that a patient with Cushing's syndrome, which is characterized by abnormally high serum concentrations of cortisol, also have hyperhomocysteinemia, which suggests that high serum homocysteine levels are a consequence of high serum cortisol levels [9].

Different results were presented by Zanardo et al. [10]. Their studies showed that the concentration of homocysteine, both in the mother's and cord blood, was higher in mothers delivering by Caesarean section in comparison with natural labor.

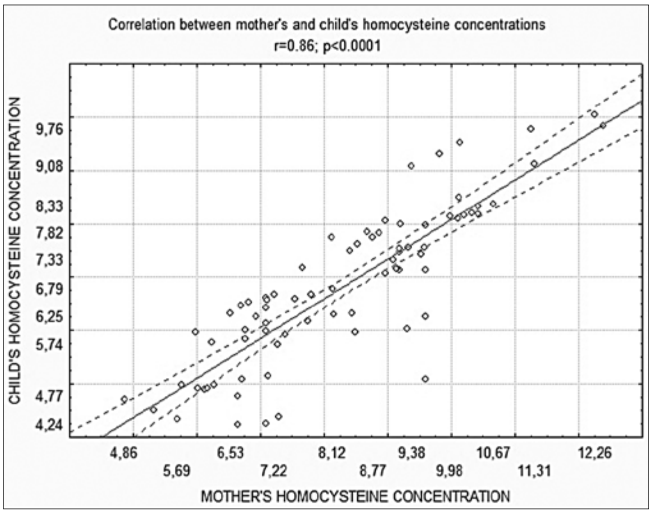

Figure 1. Correlation between mother's and child's homocysteine concentrations.

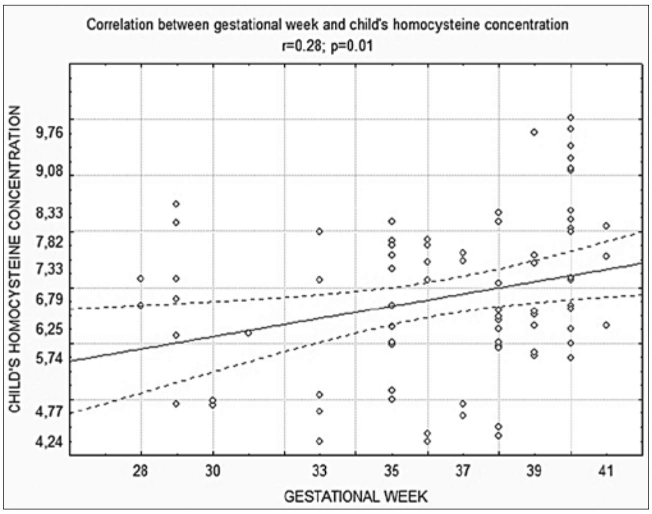

Figure 2. Correlation between gestational age and child's homocysteine concentrations.

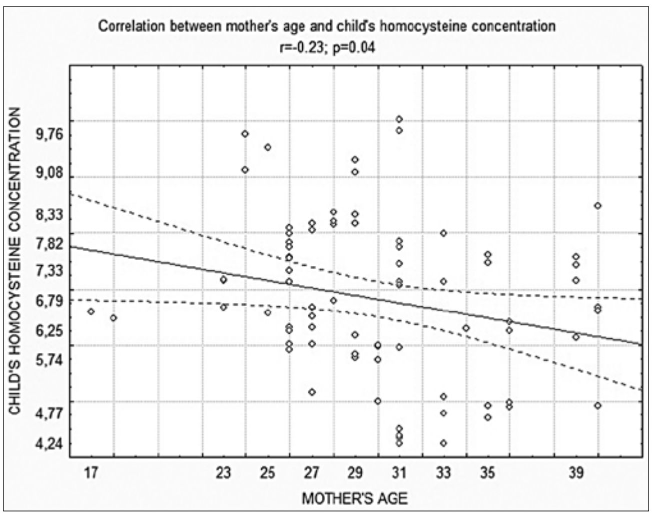

Figure 3. Correlation between mother's age and child's homocysteine concentrations.

Researchers suggested that hyperhomocysteinemia in elective Caesarean section using nitrous general anesthesia may be related to iatrogenic hormonal and/or pharmacological metabolic disturbances at the time of delivery.

Our study did not show a correlation between the concentration of homocysteine, folic acid and vitamin B12. This may result from the fact that the pregnant women, who participated in the observation, took these vitamins. There is an evident relation between the presence of folic acid, vitamin B12 and the concentration of homocysteine regardless of the mother's age [11]. Research done by Thomas et al. on a group of 12-13 year-old teenagers showed that the concentration of homocysteine, both in boys and girls, was significantly related to the concentration of folic acid, it was further observed that the concentration of homocysteine >or=8.5µmol was related to a greater chance of the occurrence of cardiovascular diseases in families [12]. In our study there were no differences in homocysteine concentration between boys and girls. But examinations of adults showed that the concentration of homocysteine is higher in men by approximately 2 µmol in comparison with women [13]. This difference is also present in the group of teenagers though not as significant as in adults [14]. This lack of differences in homocysteine levels in newborns suggests that the concentration of homocysteine related to the sex becomes present with age, which may be related to growing muscles depending on sex and age.

We also did not observe any differences in homocysteine concentration according as newborns condition.

A high concentration of homocysteine, higher than the accepted norms were observed in mothers who had different complications during pregnancy i.e. detachment of the placenta, infarct of the placenta, PROM (premature rupture of membranes) and preeclampsia [15, 16].

Our study did not confirm those observations. Mothers with hypertension did not have higher concentration of homocysteine. El Khairy et al. also observed that high concentration of homocysteine is not a risk factor of hypertension in pregnancy, pre-eclamptic state and pre-term delivery [17]. However, the researchers analysed the relation between the concentration of cysteine and homocysteine. It was found that the higher risk of the above mentioned complications is observed when a high concentration of cysteine and low concentration of homocysteine is present, and also when there is a higher concentration of both cysteine and homocysteine.

A high concentration of homocysteine can result from its defective excretion by a pregnant woman's kidneys [18]. Other researchers suggest that a high concentration of homocysteine can induce the weakening of the collagen structure through damaging transversal bindings which then leads to a weakening of connective tissue [19, 20]. This process can induce the preterm rupture of fetal membranes and cause preterm delivery. According to Kramer et al. a high homocysteine concentration may cause or be a marker for placental vascular changes that stimulate hormonal, inflammatory or cellular changes that initiate or accelerate the cascade of events leading to preterm labor or PROM [21]. A high plasma homocysteine concentration may act through a direct mechanism, however in vitro studies have reported that homocysteine increased the frequency of spontaneous contractions of a pregnant woman's myometrium suspended in an organ bath.

We conclude that determination of homocysteine level is of no significant importance in newborns pathophysiology. Homocysteine level depends on gestational age, Apgar score and mother's age. But there is no correlation between homocysteine level and hypertension during pregnancy, type of delivery, levels of vitamin B12 and foliates.

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