ORIGINAL ARTICLE

Homocysteine serum concentration and uterine artery color Doppler examination in cases of recurrent miscarriages with unexplained etiology

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Abstract

OBJECTIVES: To study the assessment of diagnostic value and clinical usefulness of the determination of homocysteine concentration in blood serum in cases of recurrent miscarriages and the relation between the concentration of homocysteine in blood serum and parameter values determining the Doppler blood flow in the uterine arteries.

METHODS: Homocysteine concentration in blood serum was determined in a group of 30 women with at least two subsequent miscarriages with no clear reason and in the control group consisted of 20 non-pregnant women without a medical history of obstetric failures, having at least one healthy child. In all cases Color Doppler sonography was performed to determine flow velocity waveforms of the uterine arteries in luteal phase of the menstrual cycle.

RESULTS: Both pulsatility (PI) and resistance indices (RI) were considerably higher ($p<0.01$, $p<0.05$) for the group of women with recurrent abortions.

In the group of women with obstetrical failures high positive correlation ($R=0.6903$, $p<0.001$) and in the control group very high positive correlation ($r=0.8163$, $p<0.001$) was found, between average values of PI and average HC concentration. High positive correlation ($R=0.6260$, $p<0.001$) in the examined group and very high positive correlation ($r=0.9201$, $p<0.001$) in the control group was obtained between average values of RI, and average HC concentrations in blood serum .

CONCLUSIONS: The recurring miscarriages occur in connection with the elevated homocysteine concentration, in consequence they can point out the pathology within the uterine-fetal blood vessels.

Abbreviations

ADP - adenosine diphosphate
HC - homocysteine
HHC - congenital hiperhomocysteinemia
MEIA - Enzyme Immunoassay method
PI - Pulsatility Index
RI - Resistance Index
TF - Tumour Factor

INTRODUCTION

Hiperhomocysteinemia (HHC) in the first trimester of pregnancy can be connected with embryo implantation disorders and clotting disorders in developing chorion. It could be related to recurrent miscarriages, ablation of the placenta or pre-eclampsia (Ray and Laskin 1999, Tug *et al*. 2003). HHC is often accompanied by congenital cardiac malformations, neural tube or other morphotic anomalies in the fetus (Cotter *et al*. 2003; Gris *et al*. 2003; Eskes 2001). The results of many studies (Chambers *et al*. 2001; Domagała *et al*. 1997; Khong 1991; Zhang *et al*. 2000) concerning the pathogenesis of atherosclerosis show the importance of the elevated homocysteine (HC) level, which is one of the factors contributing to the damage of vessel walls and the accumulation of atherosclerosis plaque. Additionally, the prothrombotic effect of HC comes mainly down to the increased activity of factors V, VII and thromboplastin. It simultaneously suppresses the expression of thrombomodulin, the binding activity of antithrombin III and tissue plasminogen activator as well as protein C activity in endothelium cells (Coppola *et al*. 2000; Domagała *et al*. 1997). The influence of HHC on platelet aggregation is also essential through the reduction of ADP activity and fibroblast stimulation and also procoagulative factor TF. It has lately been proved that HC excess can cause damage to the collagen structures of blood vessels. It can also destroy DNA cells, leading to dysfunction, and even the death of endothelium cells.

The aim of our study was the assessment of diagnostic value and clinical usefulness of the determination of HC concentration in blood serum in cases of recurrent miscarriages and determination of the relation between the concentration of HC in blood serum and parameter values determining the Doppler blood flow in the uterine arteries.

MATERIAL AND METHODS

Between February 2004 and January 2006, 30 non-pregnant women at the age from 23 to 42 (32±5.5) (group I) were diagnosed at the Department of Obstetrics and Gynaecology Medical University of Silesia, Katowice, because of two or more subsequent miscarriages. The control group (group II) consisted of 20 non-pregnant women between 22 and 40 years of age (30.5±4.5), without a medical history of obstetric failures, having at least one healthy child. The patients included into examinations (group I) were women with at least two subsequent miscarriages with no clear reason, were in relationships with the same partner. None of the women examined used hormonal therapy. Each patient agreed in writing to participate in the research. From the total number of 98 women with a medical history of obstetric failures, 68 (69.4%) were excluded from the programme (see Table 1). For the tests 5 ml of blood was taken from the antecubital vein for clot. The serum after centrifugation (3000×g;/10 min) was stored in the temperature of –200 °C. This material was transported to the Clinical Immuno-Diagnostic Laboratory Medical University of Silesia, Sosnowiec for testing. HC concentration in blood serum was determined by means of MEIA method, by using commercial Abbott sets (USA) and AIMx analyser produced by Abbott (USA). Method sensitivity is 0.50 µmol/l, producer's standard: 4.45–12.42 µmol/l and laboratory standard for healthy people: 9.62 µmol/l. Color Doppler sonography was performed to determine flow velocity waveforms of the uterine arteries in luteal phase of the menstrual cycle in a both examined groups. The determination of the speed flow in the uterine arteries was characterised by measures of Pulsatility Index (PI) and Resistance Index (RI) according to Gosling and Pourcelot. Measurements were carried out using a 5-MHz transvaginal probe (Acuson 128 XP/10; USA). Statistical analysis was performed using the Statistica PL programme. Average values and standard deviations of the examined parameters were determined. For the assessment of normal distribution Kołmogarow-Smirnow and Student's t-Test test were conducted. When there was no normal distribution, nonparametric tests were used in the analysis. In order to estimate statistical relevance of differences ANOVA, Kruskal-Wallis and Mann-Whitney U tests were done. The correlation between parameters was checked by calculating the Pearson linear correlation coefficient (normal distribution) or Spearman R coefficient (lack of normal distribution features). The frequency of the results beyond established standards of examined parameters was calculated. The percentages were compared by means of chi-square test with Yates corrections. Level $p<0.05$ was accepted as statistically relevant. The study was approved by the Bioethical Committee for Research on Humans Silesian University of Medicine, Katowice. All the women gave consent for their participation.

RESULTS

The values of HC concentration were compared in the following three ranges: ≤9.62 µmol/l (the range of laboratory standard for healthy people); >9.62 µmol/l to ≤11 µmol/l (the range above laboratory standard for healthy people and below the concentration regarded as change causing in feto-maternal circulation); >11 µmol/l (the range of HC that can cause changes in feto-maternal circulation). In the group I the average HC concentration was 9.45 µmol/l but in the group II - 8.47 µmol/l ($p>0.05$) (Table 3). Higher than 11 µmol/l HC concentrations were

more often seen (p<0.05) for women with recurrent abortions (26.7%). In group II, in each case, the obtained parameter values were below 11 µmol/l (Table 2, Table 3). HC concentrations higher than 11µmol/l in the group I were more often marked for women over 35 years of age (Table 4). Both PI and RI indices of blood flow in the uterine arteries were considerably higher (p<0.01; p<0.05) for the group of women with recurrent abortions, and their average values were PI - 2.87 and RI - 0.77 respectively (Table 5, Table 6). In the control group the average PI was 2.72, and RI - 0.70. For 50% of women from the group I the values of PI index were >2.81. Only one patient (5%) was found to have such a value in group II (p<0.01). In the group I, 26.7% of cases the RI value was lower than 0.88, but in the group II these cases were not found (p<0.05). Together with the increasing number of abortions, the average HC concentrations and the average values of PI and RI indices grew (Table 7). For the group of women who miscarried two or three times, the average HC concentrations were similar. For patients with four or more miscarriages, however, statistically relevant average HC concentrations (p<0.001) were observed in relation to the other two subgroups. The highest average values of PI were observed in the case of four or more miscarriages and they were significantly higher in comparison with the group of women with two (p<0.001) or three (p<0.05) miscarriages. No significant differences (p>0.05) were found between the subgroups of patients, who miscarried two or three times. The highest average values of RI were observed in the subgroup of women with four or more abortions. Also, in cases of three abortions, significantly higher values (p<0.01) of this parameter were found in relation to the group of women, who miscarried twice. The correlation between the average values of particular parameters and average HC concentration was also examined for groups I and II (Figure 1, Figure 2). In the group of women with obstetrical failures high positive correlation (R=0.6903, p<0.001) and in the control group very high positive correlation (r=0.8163, p<0.001) was found, between average values of average PI and average HC concentration (Figure 1). High positive correlation (R=0.6260, p<0.001) in group I and very high positive correlation (r=0.9201, p<0.001) in group II was obtained between average values of RI, and average HC concentrations in blood serum (Figure 2).

DISCUSSION

The concentration values of HC in blood serum below 10 µmol/l are regarded as safe for our health, as it was stated by the Nutrition Committee of American Cardiologist Society in 1999 (Hozyasz, 2002). There are only single reports (Chambers *et al.* 2001; Coppola *et al.* 2000; Cotter *et al.* 2003; Gris *et al.* 2003) on the behaviour of HC concentrations for physiological pregnancy. The results of the relatively few studies available are divergent (Cotter *et al.* 2003; Gris *et al.* 2003; McDonald and Walker 2001). In our research, the average HC concentration for

Table 1. The characteristics of cases excluded from the study.

Reason of exclusion	Number of cases (n=68)	(%)
Antiphospholipid antibodies presence	25	36.8
Infectious factors	15	22.1
Anatomical abnormalities	15	22.1
Chromosome aberrations	7	10.3
Immunological factors	5	7.4
Mola hydatidosa	1	1.3
Total:	**68**	**100**

Table 2. The homocysteine concentrations in blood serum in the examined group (group I) and in the control group (group II) and percentage result in the examined groups.

	Homocysteine [µmol/l]		
	≤9.62	**9.63–11**	**>11**
Examined group (n=30)	17	5	8
	56.6%	16.7%	26.7%
Control group (n=20)	18	2	0
	90.0%	10.0%	0.0%
Group comparison	**p<0.05**		

Table 3. The homocysteine concentrations in blood serum in the examined group (group I) and in the control group (group II) and percentage result in the groups examined.

	Homocysteine [µmol/l]		
	average ± SD	**≤11 µmol/l**	**>11 µmol/l**
Examined group (n=30)	9.45±2.69	22	8
		73.3%	26.7%
Control group (n=20)	8.47±1.46	20	0
		100.0%	0.0%
Group comparison	**NS**	**p<0.05**	

Table 4. The homocysteine concentration in blood serum in the examined group (group I) and in the control group (group II) considering the age barrier - 35 years in the examined group.

		Homocysteine [µmol/l]	
	Age	**≤11µmol/l**	**>11µmol/l**
Examined group (n=30)	≤35 years	19	1
		95.0%	5.0%
	>35 years	3	7
		30.0%	70.0%
Control group (n=20)		20	0
		100.0%	0.0%
Group comparison		p<0.05	

Table 5. The average values of pulsatility index (PI) of blood flow in the uterine arteries in the examined group (group I) and in the control group (group II). The number of cases, in which the value of PI was lower or equalled 2.81 and higher than the limit value.

	Pulsatility index (PI)		
	average ± SD	**≤2.81**	**>2.81**
Examined group (n=30)	2.87±0.19	15 50.0%	15 50.0%
Control group (n=20)	2.72±0.08	19 95.0%	1 5.0%
Group comparison	**p<0.01**	**p<0.01**	

Table 6. The average values of resistance index (RI) of blood flow in the uterine arteries in the examined group (group I) and in the control group (group II). The number of cases in which the value of RI was lower or equalled 0.88 and higher than the limit value.

	Resistance index (RI)		
	Average ± SD	**≤0.88**	**>0.88**
Examined group (n=30)	0.77±0.11	22 73.3%	8 26.7%
Control group (n=20)	0.70±0.09	20 100.0%	0 0.0%
Group comparison	**p<0.05**	**p<0.05**	

Table 7. The average values of parameters assessed depending on the abortion number in the examined group (group I).

	Abortion number			**ANOVA**
	Two (n=13)	**Three** (n=6)	**Four or more** (n=11)	Kruskal-Wallis Test
Homocysteine [μmol/l]	7.76±1.23	7.75±0.78	12.35±2.05	p<0.001
Pulsatility index (PI)	2.74±0.09	2.82±0.11	3.05±0.16	p<0.001
Resistance index (RI)	0.66±0.05	0.77±0.08	0.89±0.05	p<0.001

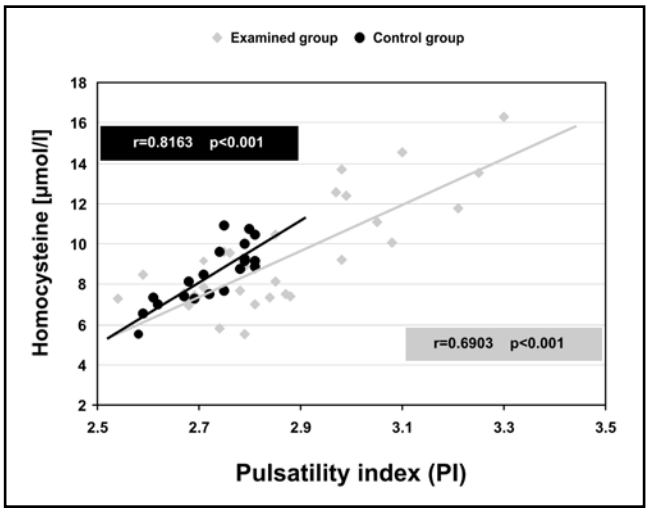

Figure 1. The correlation between the average values of pulsatility index (PI), and average homocysteine concentrations in blood serum in the examined group (group I) (n=30) and in the control group (group II) (n=20).

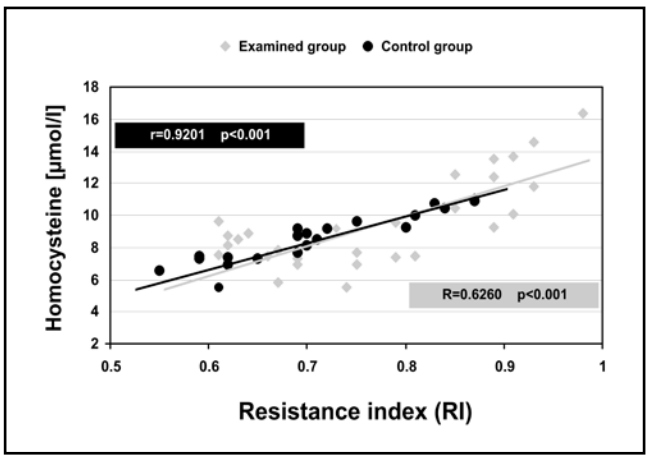

Figure 2. The correlation between the average values of resistance index (RI), and average homocysteine concentrations in blood serum in the examined group (group I) (n=30) and in the control group (group II) (n=20).

patients without obstetrical failures was 8.47 μmol/l and it was statistically insignificantly lower compared to patients with recurrent abortions (9.45 μmol/l). In the group I, a significant increase of average HC concentration was observed in the subgroup of four or more abortions, in comparison with cases of two or three pregnancy losses in medical history. As it results from the research presented, the elevated HC concentration occurred considerably more frequent in cases of recurrent abortions (26.7%), and concentration values above 9.62 μmol/l were more often observed for women with recurrent miscarriages, which is true with the reports of McDonald and Walker (2001), who showed that for 20% of recurrently miscarrying women the elevated HC level can be observed. However, these authors took a considerably lower limit value, estimating HC standard at the level of 5.6μmol/l (Łopaciuk, 2002; McDonald &Walker, 2002). So far, no unequivocal answer has been given, if in case of HHC the cause of abortions can certainly be embolic vascular changes limiting blood flow within uterine blood vessels and chorion. In the references (Khong, 1991; McDonald &Walker, 2002), only a few studies dealing with changes in decidual vessels in early pregnancy were found. In early pregnancy, i.e. till the eighth week, pathological changes in the decidua are rather rare (Nadji &Sommers, 1973). The elevated HC concentrations simultaneously cause damage in blood vessel collagen and they can destabilise the structures of cellular DNA, leading to dysfunctions, and

even death of endothelium cells (Eskes, 2001; Hozyasz, 2002; Wilson *et al*. 1999). In the Steer and co-authors research (Steer *et al*. 1992) it was claimed that excessive value of PI above 3.0 caused failures of embryo implantation and growth. The highest endometrium ability for implantation is when the value of PI ranges between 2.00 and 2.99. Battaglia *et al.* (1998) did not gain any pregnancies in the group of women, in which the PI was above 3.0. It can be therefore claimed that the decrease of peripheral resistance in the uterine vascular placenta, which is characteristic for low value of PI is connected with the growth of blood flow and better perfusion, which has the influence on better implantation ability of the uterine body mucosa. In our research, significantly statistically higher average values of PI in the uterine arteries for patients with the elevated HC concentrations in blood serum (PI - 2.87) were showed, in comparison with women from the control group (PI - 2.72). Similar results were obtained by Battaglia (1990; 1998). It should, however, be emphasised that in the study quoted, the measurements were taken on the day of embryo transfer, but in the present research the analysis was conducted in the menstrual phase II (6^{th}–8^{th} day after ovulation). The comparison of the obtained average values of RI showed statistically significant differences between the assessed groups (0.77–0.70). No information in references was found about the comparison of RI values for recurrently miscarrying women with disorders of HC metabolism. In Tsai *et al.* (2000) study, the significance of blood flow determination parameters in the prediction of pregnancy success was not confirmed. The obtained average values of PI and RI were respectively 2.67 and 0.72 in the group of patients who became pregnant, and 2.81 and 0.88 for women whose treatment failed. A question, therefore must be asked, if it is possible to determine such a limit value of PI and RI that would correlate with the increasing HC level in blood serum? It seems that determinations of HC concentration are the next stage in acknowledging the etiology of recurrent abortions. In future, they can be the next necessary step for therapeutic success in this group of patients. Establishing the reason of recurrent abortions and appropriate treatment is not always the only condition to achieve the goal, which is delivery of a healthy child. The last investigation (Jivraj *et al*. 2001) shows that women with recurrent miscarriages in medical history are a group of high risk of obstetric complications. They, therefore, need special care not only in the period of pre-conception but also in the next pregnancy course and in the perinatal period.

CONCLUSIONS

1. The recurring miscarriages occur in connection with the elevated homocysteine concentration, in consequence they can point out the pathology within the uterine-fetal blood vessels.
2. Disorders of homocysteine metabolism can be observed in women with four or more miscarriages.

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