Homocysteine, folic acid and vitamin B_{12} concentration in patients with recurrent miscarriages

Jerzy Sikora[1], Jacek Magnucki[1], Jerzy Ziętek[1], Lucyna Kobielska[1], Robert Partyka[2], Danuta Kokocinska[2], Aleksandra Białas [1]

1. Department of Obstetrics and Gynaecology, Medical University of Silesia, Katowice, Poland
2. Independent Clinical Immunodiagnostic Laboratory, Medical University of Silesia, St. Barbara's Hospital, Sosnowiec, Poland

Correspondence to: Jerzy Sikora, Assistant Professor
Head of Department of Obstetrics and Gynaecology, Silesian University of Medicine
ul. Medyków 14, 40-752 Katowice, Poland
PHONE: +48 (032) 789 47 01; +48 (032) 25 25 302
FAX: +48 (032) 25 25 302
EMAIL: jerzy_sikora@poczta.onet.pl; j.sikora@csk.katowice.pl

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Abstract

OBJECTIVES: The aim of the project was the assessment of clinical usefulness of the determination of blood serum homocysteine concentration, folic acid and vitamin B_{12} in recurrent miscarriages.

METHODS: 30 non-pregnant women with recurrent miscarriages (examined group - I) and for 20 non-pregnant women without obstetric failures in medical history (control group- II) were examined.

RESULTS: In the examined group (group I), the average concentration of homocysteine (9,45μmol/l) was not statistically higher in comparison to the control group (group II) (8,47μmol/l) ($p>0,05$). In group I the average vitamin B_{12} concentration in blood serum was 178,3pg/ml and it was statistically lower ($p<0,001$) in comparison with the control group (II) (268,6pg/ml). Such a relation was not observed for the vitamin B_{12}, where the average concentration of this parameter was not dependant on the miscarriage number. A high negative correlation ($R= -0,5397$, $p<0,01$) was observed between the level of folic acid and homocysteine concentration in the group of women with recurrent miscarriages and a very high negative correlation ($r= -0,9586$ $p<0,001$) in the control group. No relation ($R=0,0992$ $p>0,05$) between the average concentration of vitamin B_{12} in blood serum and the average homocysteine in the nullipara group with recurrent miscarriages.

CONCLUSIONS: Together with the increasing number of abortions, the average homocysteine concentrations grew and the average folic acid concentrations lowered.

ORIGINAL ARTICLE

Abbreviations

CBS – cystationine-ß-synthetase
HC – homocysteine concentration
HHC – congenital hiperhomocysteinemia
MEIA – Enzyme Immunoassay method
MTHFR – methylenetetrahydrofolate reductase
PROM – prolonged rupture of membranes

INTRODUCTION

At the end of the previous century it was noticed for the first time that in pregnant women with elevated homocysteine concentration (HC) often comes to recurrent miscarriages, premature placental abruption, preeclampsia or prolonged rupture of membranes (PROM), whereas in fetus cardiac and neural tube defects are more often found (Cotter et al. 2003; Eskes, 2001; Gris et al. 2003; Rozen, 2000; Wenstrom et al. 2001; Tug et al. 2003).

Congenital hiperhomocysteinemia (HHC) is caused by enzymatic block of methionine-cysteine biochemical cycle and it can result from the deficiency of appropriate enzymes or can be the effect of genetic mutation lowering its activity (Cichocka et al. 1999; Fattal Valevski et al. 2000; Jacobsen, 1998). These enzymes are: cystationine-ß-synthetase (CBS), methylenetetrahydrofolate reductase (MTHFR) and methionine synthetase (Domagała et al. 1997). In 1988 a thermolabile form of MTHFR was discovered connected with the C677T presence of MTHFR gene causing the change of alanine into valine in enzyme molecule (Domagała et al. 1997; Jacobsen, 1998; Malinow et al. 1997). An abnormal enzyme becomes thermolabile and shows and increased affinity for folates, which results in moderate hiperhomocysteinemia (Koch, 2000; Ray and Laskin, 1999).

Secondary hiperhomocysteinemia is caused by different factors, which influence the elevated HC level in blood serum. Among them there are: folic acid and vitamin B_6 and B_{12} deficiency, overconsumption of meat and its products, the elevated level of creatinine in blood serum, as well as smoking (Koch, 2000). There is a negative feedback between folic acid level and HC in blood serum.

In women with hiperhomocysteinemia it more often comes to embryo implantation disorders and clotting disorders in developing chorion, which can be a consequence of miscarriages. In women with more miscarriages a positive result of methionine load test and elevated homocysteine concentration can be observed (McDonald and Walker, 2001). A correlation between the polymorphism of MTHFR C677T and A1298C genes and the risk of recurrent miscarriages in an early pregnancy was also shown (Chambers et al. 2001; Coppola et al. 2000).

Recurrent miscarriages can be defined as three or more subsequent pregnancy losses for women being in the same relationship (Collins &Roberts 1997). In the clinical practice however, already two subsequent spontaneous abortions can be qualified as recurrent and appropriate diagnostic and therapeutic procedures can be applied (Coulam,1991). Among the most common reasons for recurrent miscarriages are the following: genetic factors, anatomic defects of the uterus, metabolic, endocrinological and immunological disorders as well as infections. It has been lately claimed that the elevated homocysteine concentration has a negative influence on the development of an early pregnancy. It was shown that the level of HC could be harmless for endothelium vessels in the range of 11-13µmol/l (Koch, 2000). The concentration values of HC in blood serum below 10µmol/l are regarded as safe for our health, as it was stated by the Nutrition Committee of American Cardiologist Society in 1999. However, there are still divergences concerning values of homocysteine concentrations. The biggest problem is the border between normohomocysteinemia and mild hiperhomocysteinemia. The range of HC concentrations in blood serum is also not known in women with recurrent miscarriages in medical history.

The aim of the project was the assessment of clinical usefulness of the determination of homocysteine concentration, folic acid and vitamin B_{12} for women with recurrent miscarriages and the determination of the correlation between the examined parameters.

MATERIALS AND METHODS

30 non-pregnant women were qualified for the examinations, aged 23-42, (average 32,1±5,5) diagnosed in Department of Obstetrics and Gynaecology, Medical University of Silesia, Katowice, Poland, because of two or more subsequent miscarriages with no clear reason. The examined patients were in relationship with the same partner. The control group consisted of 20 non-pregnant women, aged 22-40 (average 30,5 ± 4,5), without obstetric failures in medical history, having at least one healthy child. These groups, taking into account age criterion, were homogeneous. None of the women examined used hormonal therapy. The study was approved by the Bioethical Committee for Research on Humans Silesian University of Medicine, Katowice. Each patient agreed in writing to participate in the research. The following criteria of exclusion were used:

- anatomical abnormalities within reproductive organs,
- chromosome aberrations in a patient or her partner,
- infectious factors (TORCH, Chlamydia Trachomatis, Bacterial Vaginosis) or inflammatory condition within cervical canal,
- positive sperm culture in a partner,
- incorrect results of histopathological examination of the material obtained after miscarriage,
- miscarriage cases of unknown etiology in which essential suspicion of alloimmunological background existed,
- cases in which antiphospholipid syndrome was discovered,

- folic acid supplementation during last three months,
- withdrawal of an earlier agreement to take part in the examinations.

Homocysteine concentration in blood serum was determined by means of Microparticle Enzyme Immunoassay method (MEIA), using commercial Abbott sets (USA) and AIMx analyser by Abbott (USA). Method sensitivity was below 0,50μmol/l, the producer's standard: 4,45–12,42μmol/l and laboratory standard for healthy people: 8,47μmol/l. The concentration of folic acid was also determined according to MEIA method, using commercial Abbott sets (USA) and Axym apparatus by ABBOTT (USA). The normal set values were 3- 17 ng/ml, and the standard was- 9,28 ng/ml.

The determination of vitamin B_{12} concentration was performed according to immunoenzymatic method (MEIA), using commercial Abbott sets (USA) and Axym analyser by ABBOTT (USA). The producer's standard: 180-800pg/ml. Laboratory standard: 268,6pg/ml.

Statistical calculations were taken by means of STATISTICA PL programme. Average values and standard deviations of the examined parameters were determined. For the assessment of normal distribution Kołmogarow-Smirnow test was conducted. In the case of population with normal distribution comparisons Student's t-Test was used. 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. The level $p<0,05$ was accepted as statistically relevant.

RESULTS

In the examined group the average concentration of homocysteine was 9,45μmol/l and in the control group– 8,47μmol/l. This difference, however, was not statistically relevant ($p>0,05$). Higher homocysteine concentrations were more often ($p<0,05$) seen for women with recurrent miscarriages (26,7%), in comparison with the control group, in which the obtained parameter values were below 11μmol/l in each case (Table 1). Statistically relevant higher values of folic acid ($p<0,001$) were observed in the women group with recurrent miscarriages compared to the control group and they were 13,21ng/ml and 9,28 ng/ml respectively. For 23,3% of women from the examined group the elevated level of folic acid was observed and this percentage was statistically relevant ($p<0,05$) in comparison with the women from the control group, where no elevated values of this parameter were observed (Table 2). For the group of women who miscarried two or three times, the average homocysteine concentrations were similar. For patients with four or more miscarriages, however, statistically relevant ($p<0,001$) higher homocysteine concentrations were observed in relation with the other two subgroups. In nullipara group with recurrent miscarriages in medical history (group I), the average vitamin B_{12} concentration in blood serum was 178,3pg/ml and it was statistically lower ($p<0,001$) in comparison with the control group (268,6pg/ml).

The percentage of women with vitamin B_{12} concentration ≤180pg/ml was significantly higher in the examined group and it was 40%. In the control group no vitamin B_{12} concentration ≤180pg/ml was observed (Table 3).

The average homocysteine and folic acid concentration underwent essential changes with the number of abortions. Together with the increasing number of abortions, the average homocysteine concentrations grew and the average folic acid concentrations lowered. Such a relation was not observed for the vitamin B_{12}, where the average concentration of this parameter was not dependant on the miscarriage number (Table 4).

Table 1. The average homocysteine concentration in the examined group (n=30) and in the control group (n=20)

Group	Homocysteine concentration [μmol/l]		
	Average value ± SD	≤11 μmol/l	> 11 μmol/l
Examined	9,45 ± 2,69	22 (73,3%)	8 (26,7%)
Control	8,47 ± 1,46	20 (100,0%)	0(0,0%)
Group comparison	NS	p < 0,05	

Table 2. The average folic acid concentrations in the examined group (n=30) and in the control group (n= 20)

Group	Folic acid concentration [ng/ml]		
	Average value ± SD	≤17 ng/ml	> 17 ng/ml
Examined	13,21 ± 5,83	23 (76,7%)	7 (23,3%)
Control	9,28 ± 1,74	20 (100,0%)	0 (0,0%)
Group comparison	p < 0,001	p < 0,05	

Table 3. The average concentration values of vitamin B_{12} in the examined group (group I) (n=30) and in the control group (group II) (n=20)

Group	Vitamin B_{12} [pg/ml]		
	average ± SD	≤180 pg/ml	>180 pg/ml
Examined	178,3 ± 60,5	12	18
		40,0%	60,0%
Control	268,6 ± 50,1	0	20
		0,0%	100,0%
Group comparison	p<0,001	p< 0,001	

Table 4. The average values of the assessed parameters in relation with the number of miscarriages in the examined group (group I) (n=30)

	Miscarriage number			**ANOVA Kruskal-Wallis test**
	Two (n=13)	**Three (n=6)**	**Four or more (n=11)**	
Homocysteine [µmol/l]	7,76 ± 1,23	7,75 ± 0,78	12,35 ± 2,05	p<0,001
Folic acid [ng/ml]	16,8 ± 6,2	13,6 ± 4,3	8,8 ± 1,9	p<0,001
Vitamin B_{12} [pg/ml]	176,4 ± 69,3	174,8 ± 75,9	182,3 ± 44,2	NS

For the group of women who miscarried two or three times, the average homocysteine concentrations were similar. For patients with four or more miscarriages, however, statistically relevant higher average HC concentrations (p<0,001) were observed in relation to the other two subgroups (Table 4).

The highest average concentration of folic acid in blood serum was observed in the group of women with two miscarriages in comparison with the result from the other two subgroups. Only in relation with the subgroup of women with four or more miscarriages, the difference was statistically relevant (p<0,001). A statistically relevant difference (p<0,05) between the average folic acid concentration obtained in the subgroups of women with three and four or more miscarriages was also determined (Table 4). In all cases of recurrent miscarriages the average concentrations of vitamin B_{12} were similar, and the differences among them were not significant (p>0,05) (Table 4).

A high negative correlation (R= -0,5397, p<0,01) was observed between the level of folic acid and homocysteine concentration in the group of women with recurrent miscarriages and a very high negative correlation (r= -0,9586 p<0,001) in the control group (Figure 1). No relation (R=0,0992 p>0,05) between the average concentration of vitamin B_{12} in blood serum and the average homocysteine in the nullipara group with recurrent miscarriages. A very high negative correlation (R =-0,9501 p<0,001) was noticed in the control group (Figure 2).

DISCUSSION

According to the WHO definition, recurrent miscarriage can be defined as three or more subsequent pregnancy losses. We also included patients with two subsequent miscarriages in our research. Then, appropriate diagnostic and therapeutic procedures can be applied, which can improve the prognosis for the next pregnancy (Malinow et al. 1997; Klimek et al. 2005). The elevated homocysteine concentration can be one of the reasons for recurrent abortions. Despite numerous examinations of different negative effects of the high level of homocysteine in the human organism, up till now no specific critical concentration values of this amino acid in blood serum have been determined, above which pathological vascular changes can be caused, and in consequence pregnancy loss. The appropriate concentration of folic acid and vitamin B_{12} in the pregnant woman organism is also very significant for pregnancy growth. Hibbard (Steegers et al. 1992) was the first to notice the disorders of folates matabolism and hiperhomocysteinemia in women with recurrent miscarriages. Then, a hypothesis was formulated that even the moderate elevation of HC concentration can disturb the proper embryogenesis (Steegers et al. 1992). Among the results of hyperhomocysteine there are changes in endothelium of the blood vessels, with accompanying irregularities in blood coagulation system. According to many clinical reports, dealing with the pathology of the first pregnancy trimester, the normal folic acid concentrations in blood serum range from 4,0 ng/ml to 17ng/ml. Clarke (1999) analysing 12 randomised examinations concerning the influence of folic acid supplementation on HC concentration, tried

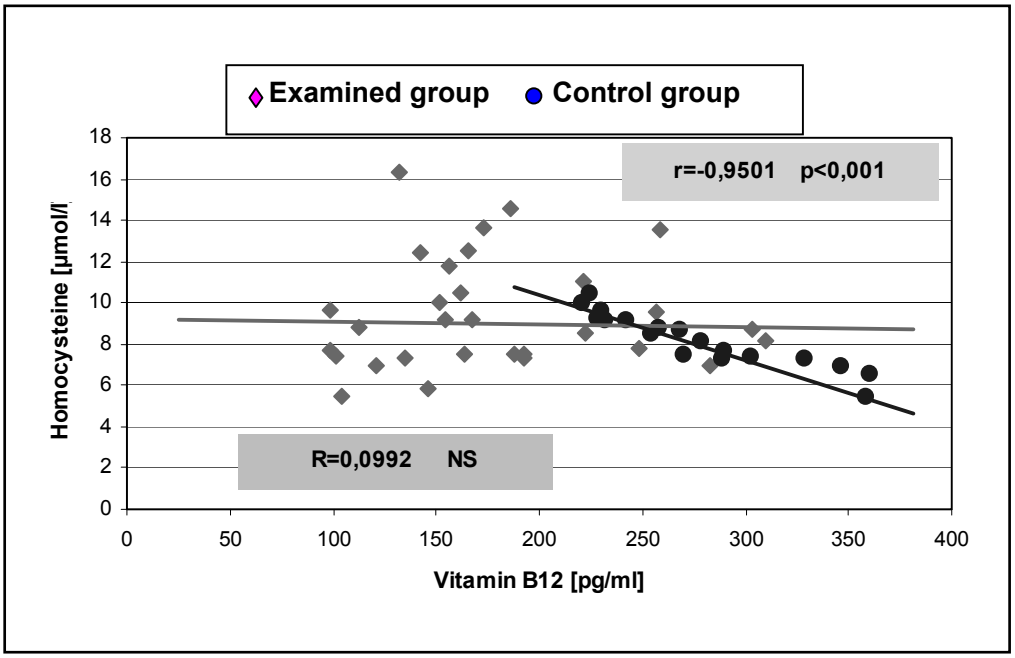

Figure 2. The relation etween the average concentration of vitamin B12 in blood serum and the average homocysteine concentration in the examined group (group I) (n=30) and in the control group (group II) (n=20).

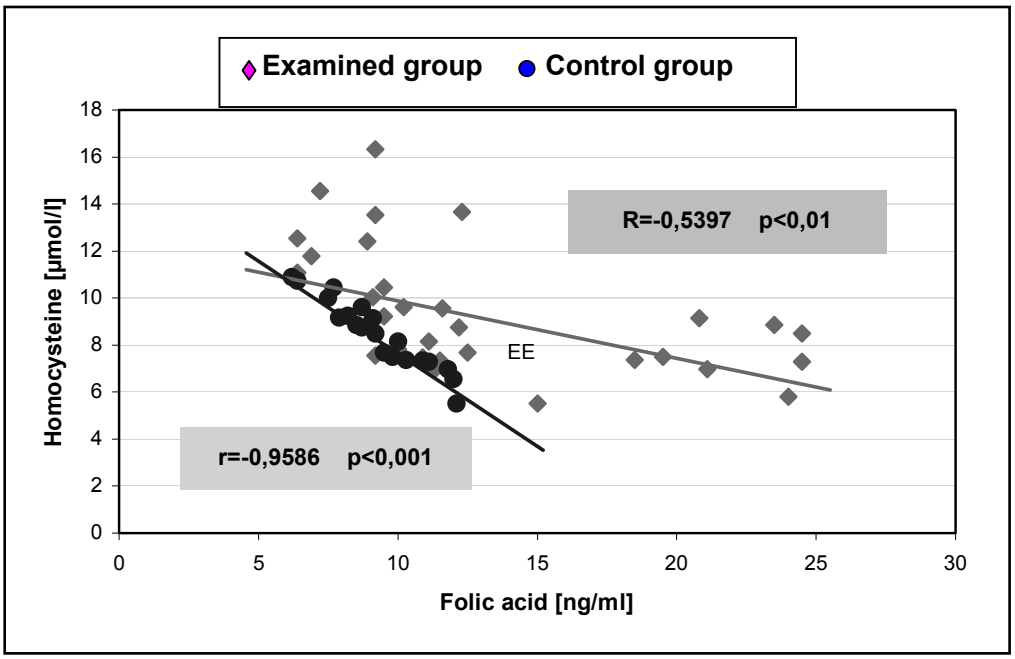

Figure 1. The relation between the average folic acid concentration in blood serum and the average homocysteine concentration in the examined group (group I) (n=30) and in the control group (group II) (n=20).

to determine the average concentration of folic acid in blood before the treatment. The dispersion of values given by different authors (Clarke, 1999) is interesting. They range from 4,7 nmol/l to 23 nmol/. The decrease level of homocysteine concentration in blood serum is connected with age and the time of folic acid supplementation. It is not related, however, with vitamin B_{12} concentration, unless it is applied together with folates (Clarke, 1999). Daily doses of folic acid 0,5-5 mg lower the homocysteine concentration by about 20%, and what is interesting, the higher HC and lower folic acid concentration the bigger HC decrease under the influence of additional folates supplementation. Similar observations were conducted by Brattström et al.(1988) and Malinow et al. (1997). These authors suggest that even a daily folic acid dose of 400 µg lowers the HC concentration in blood. It seems that in cases of recurrent miscarriages, caused by homocysteine matabolic disorders, therapeutic doses of folic acid together with vitamin B_6 and B_{12} should be applied in the pre-contraception period and during the next pregnancy. Simultaneously, in each case the application of antithrombotic drugs (acetylsalicylic acid derivatives, low-molecular-weight heparin) should be considered.

CONCLUSIONS

1. In women with four or more miscarriages it probably comes to disorders of homocysteine metabolism.
2. Recurrent miscarriages can be connected with the elevated homocysteine concentration.
3. Folic acid deficiency can be also related with recurrent miscarriages.
4. In cases of recurrent pregnancy losses no correlation between plasmatic concentration of vitamin B_{12} and homocysteine concentration was determined.

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