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Homocysteine and recurrent miscarriage

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Abstract

Background: It is known that etiological structure of recurrent miscarriage has genetic, anatomical, infectious and immunological factors; however, the cause of recurrent miscarriage in 50-60% of cases is not completely clear. Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine. Development mechanisms of vascular complications of hyperhomocysteinemia are currently being intensively studied. Hyperhomocysteinemia affects a number of mechanisms involved in thrombogenesis including coagulation cascade, vessel-thrombocytic section, oxidation-reduction reactions, endothelium, and vascular smooth muscle cells and is associated with an increased risk of adverse outcomes in pregnancy.
Materials and methods: The study included 50 women who had experienced the loss of at least two consecutive pregnancies. The level of the total serum homocysteine was measured via the chemiluminescent method.
Results: We found that plasma homocysteine concentration < 10 μmol/l was found in 16 patients (32,0%, 95% CI 19,07 – 44,93), 9 patients (18.0%, 95% CI 7,36 – 28,64) had a fasting plasma homocysteine between 10 μmol/l to 12 μmol/l and 25 patients (50.0%, 95%CI 36,15 – 63,85) had significantly high total serum homocysteine values. Among them, 23 patients (46.0%, 95%CI 32,19 – 59,81) had the concentration between 12 – 30 μmol/l and 2 patients (4,0%, 95%CI -1,43 – 9,43) had the concentration > 30μmol/l. The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception. In the current study, 40 women (80.0%, 95% CI 68,92 – 91,08) have become pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.
Conclusions: The prevalence of hyperhomocysteinemia was more in unexplained primary early recurrent miscarriages. The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception and 40 women (80.0%, 95% CI 68,92 – 91,08) became pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.
Key words: homocysteine, spontaneous abortion, recurrent abortion, vitamin B supplementation.

Introduction

Spontaneous abortions (SA) are one of the most frequent pregnancy disorders and are defined as the loss of fetal product before 22 weeks of gestation [2]. SA is observed in approximately 15-20% of total pregnancies [3], although this proportion could even be higher. Roughly 1 in 5 pregnancies would, thus, end in spontaneous abortion, the majority until the gestational age of 12-14 weeks, and 1-2% would account for recurrent spontaneous abortions [2]. Recurrent miscarriage (RM) is defined as the occurrence of three or more consecutive losses of pregnancy [5]. However, many clinicians define RM as two or more losses and this increases the percentage of RM from 1% to 5% of all couples trying to conceive. RM is a very frustrating condition for both the couple and the clinician, because it is difficult to find a distinct reason for the repeated failure to sustain a pregnancy and eventually have a successful pregnancy outcome. The causes involved in RM are numerous: anatomical, endocrine, immunological, infectious, environmental, and genetic or combinations of these [3]. Not infrequently, despite all medical advances, these causes cannot be identified with certainty, and the abortion declared idiopathic remains the final diagnosis in approximately 50% of cases [2].

During the past decade, the list of candidate causes of recurrent pregnancy loss (RPL) has grown rapidly. During the early 1990s, an elevated plasma homocysteine concentration, which has been described as a risk factor for arteriosclerosis, venous thrombosis, neural tube defects, placental abruption or infarction and preeclampsia, was also suggested to be associated with RPL [8,10].

Several reports have clearly shown an association of elevated homocysteine concentrations and obstetric diseases that are connected with vascular disorders of pregnancy or of the utero-placental unit [3].

Nelen et al. [8] studied women with repeated miscarriages and found a direct relationship between high levels of homocysteine and defective chorionic villous vascularization: early miscarriages might be explained by the damage that excess homocysteine may cause on chorionic and decidual vessels leading to defective implantation of the embryo. Placental development in early pregnancy may be negatively influenced by increased maternal homocysteine concentrations [1]. Experimental studies revealed that moderately elevated homocysteine concentrations may induce cytotoxic and oxidative stress, leading to endothelial cell impairment [1, 6, 13, 16]. Additionally, exposure of trophoblast cells to homocysteine may increase cellular apoptosis and lead to inhibition of trophoblastic function [1,3]. Recently, Di Simone et al. [3] provided the first demonstration that human placenta is a target for homocysteine and suggested that trophoblast death might represent one mechanism by which homocysteine causes pregnancy complications related to placental diseases.

The aim of this study was to evaluate the level of plasma homocysteine concentration in a group of women with recurrent pregnancy loss and to investigate the association between them in order to look for hypothetical possibilities of therapeutic interventions.

Material and methods

The study included 50 women of fertile age with at least two consecutive miscarriages in the first and second trimester of gestation. All the enrolled cases have no identified causes of abortions. The patients were recruited at the Department of Obstetrics and Gynecology No 2, Nicolae Testemitsanu State University of Medicine and Pharmacy and at the medical center "Repromed"of Chisinau city, the Republic of Moldova from April to September in 2014 to 2016. The cases were identified and selected when they visited the above-mentioned hospitals for investigation of two or more consecutive unexplained terminations of pregnancy. Women with clinical diagnosis of metabolic deseases, renal insufficiency and a woman who took vitamin supplements were excluded from this study. Socioeconomic data (schooling and occupation) and obstetrics data were assessed by questionnaire. Written informed consent was obtained from all women before participation. The study was approved by the Ethics Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova.

The measurements of plasma homocysteine concentration were performed using chemiluminescence method. Blood samples were drawn from women using sterile tubes with anticoagulant (EDTA) and the plasma for the analysis of total homocysteine concentration was centrifuged immediately.

The lowering homocysteine level therapy included vitamin supplementation (folic acid, vitamin B12 and vitamin B6) and was also recommended to eat a diet rich in vitamins, as a part of preconception care.

The Microsoft Excel application (Microsoft Office 2010) and online OpenEpi application, version 2.3.1 [9] were used for the statistical analysis. Parametric variables were compared by Student's *t*-test and the 95% confidence interval (CI) was calculated.

Results and discussion

The median age of the 50 women in the study group was 29,52±5.89 years, ranging from 20 to 42 years old. The documentation of patients' places of residence identified that 27 respondents (54,0%, 95% CI 40,19 – 67,81) were from urban area and 23 respondents (46,0%, 95% CI 32,19 – 59,81) were from rural area. The study has included 4 students (8,0%, 95% CI 0,49 – 15,51), 28 respondents (56,0%, 95% CI 42,25 – 69,75) were engaged in an intellectual type of work, 13 respondents (26,0%, 95% CI 13,49 – 38,51) were workers and 5 respondents (10,0%, 95% CI 1,69 – 18,31) performed housework.

The gestational age, when the abortions occurred, was divided into the first and the second trimester of gestation and mixed when there were intersessions between them. Our data show that in 44 women (88,0%, 95% CI 79,0 – 97,0), the abortion occurred in the first trimester, in 1 woman (2,0%, 95% CI -1,43 – 9,43) 22 (25%) the abortion occurred in the second trimester and 5women (10,0%, 95% CI 1,69 – 18,31) were included in the mixed abortion group, because they presented these events in the first and second trimester during their pregnancies (fig. 1). We can conclude that 49 patients (98,0%, 95% CI 94,12 – 101,88) had early RPL and 6 patents (12,0%, 95% CI 3,0 – 21,0) had late RPL.

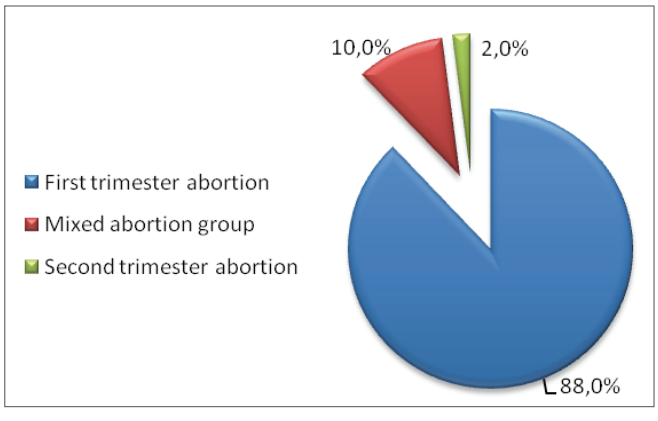

Fig. 1. The gestational – age distribution of spontaneous abortion in the study (%).

The median abortion number in the study group was 2,32±0,62, ranging from 2 to 5 abortions, in which 37 women (74,0%, 95% CI 61,85 – 86,15) had two spontaneous abortions; 11 (22,0%, 95% CI 10,52 – 33,48) of them had three spontaneous abortions, 1 (2,0%, 95% CI -1,43 – 9,43) of them had four spontaneous abortions and, also, 1 (2,0%, 95% CI -1,43 – 9,43) of them had five spontaneous abortions (fig. 2).

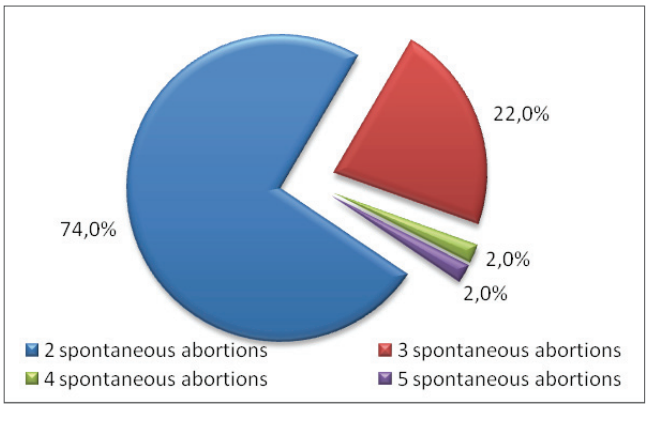

Fig. 2. The frequency of spontaneous abortions in the study (%).

Also, 33 patients (66,0%, 95% CI 52,87 – 79,13) were classified as suffering from primary RPL when they had never had a live birth before and 17 patients (34,0%, 95% CI 20,87-47,13) suffering from secondary RPL when they had recurrent losses following a successful pregnancy (fig. 3).

The median fasting total plasma homocysteine concentration was 13,36±1,0 μmol/l, ranging from 2,19 mmol/l to

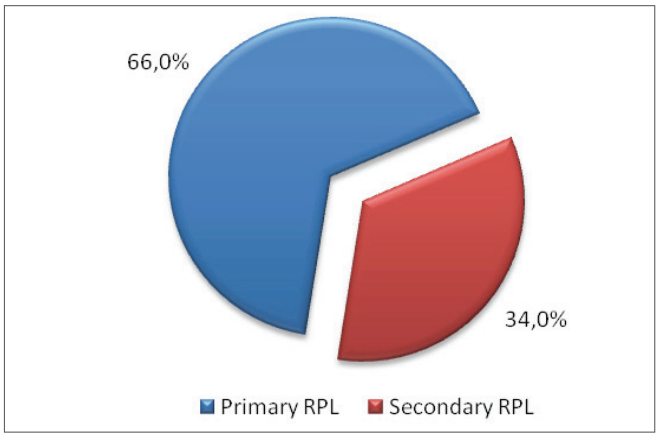

Fig. 3. Type of pregnancy loss in recurrent miscarriage in the study (%).

34,7 mmol/l.

According to the D.A.CH. – Liga Homocysteine (German, Austrian, and Swiss Homocysteine Society) fasting total serum homocysteine (< 10 μmol/l) is considered safe and should be the target level during homocysteine-lowering treatment [13]. Vollset et al. [16] reported that the women with the highest level of homocysteine (greater than 10 μmol/l) have an adjusted risk for preeclampsia. Ronnenberg et al. [12] demonstrated that the risk of preterm birth was nearly 4-fold higher among women with preconception homocysteine concentrations ≥ 12.4 μmol/l compared to women who had lower homocysteine concentrations. Urban et al. [15] mentioned that the mean serum homocysteine concentration was 11,50 μmol/l in the group of patients with intrauterine fetal growth restriction compared to the group of normal pregnacies who had lower homocysteine concentrations – 9,58 μmol/l.

Taking in consideration the information mentioned above, for these analyses, we defined elevated homocysteine as a plasma concentration of homocysteine > 10 μmol/l.

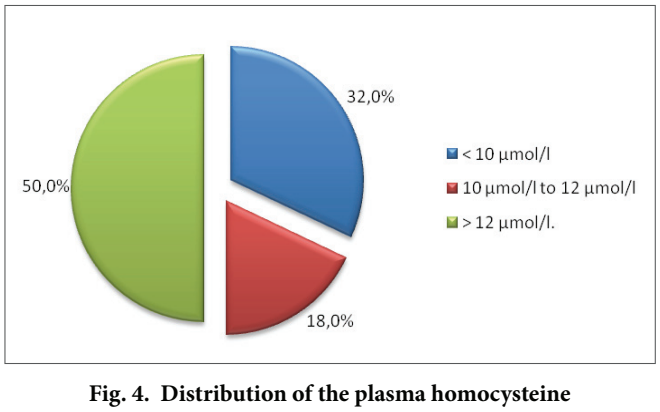

Fig. 4. Distribution of the plasma homocysteine level in the study group (%).

The results of our investigations showed that plasma homocysteine concentration < 10 μmol/l was found in 16 patients (32,0%, 95% CI 19,07 – 44,93), 9 patients (18,0%, 95% CI 7,36 – 28,64) had a fasting plasma homocysteine between 10 μmol/l to 12 μmol/l and 25 patients (50,0%, 95%CI 36,15 – 63,85) had significantly high total serum homocysteine values (fig. 4). Among them, 23 patients (46,0%, 95%CI 32,19 – 59,81) had the concentration between 12 – 30 μmol/l and 2 patients (4,0%, 95%CI -1,43 – 9,43) had the concentration > 30μmol/l.

Ueland et al. suggested that the correlation between preconceptional total serum homocysteine and total serum homocysteine during pregnancy points to the possibility that preconceptional total serum homocysteine may predict homocysteine-associated pregnancy complications [14]. As a result, we defined that 34 patients (68,0%, 95% CI 55,07 – 74,93) had elevated serum homocysteine (higher than 10 μmol/l, according to our initial consideration).

In this context, it was evaluated total plasma homocysteine concentration in relation to the type of recurrent miscarriage in women with unexplained pregnancy loss (tab. 1).

Table 1

Total plasma homocysteine concentration in relation to the type of recurrent miscarriage in the study group

Total plasma homocysteine concentration (μmol/l)	Primary RPL	Secondary RPL	t	p
	14,48±1,49	11,55±0,94	1.6631	>0.05

The results of our investigations showed that homocysteine levels in the blood serum of patients suffering from primary RPL was higher (14,48±1,49 μmol/l) compared to patients suffering from secondary RPL (11,55±0,94 μmol/l) (p >0.05).

We found that the total serum homocysteine concentration in the study group was higher (13,50±1,01 μmol/l) in the patients with a history of early RPL (tab. 2) than in the patients with a history of late RPL (9,98±1,69 μmol/l) (p >0.05).

Table 2

Total plasma homocysteine concentration according to the stage of pregnancy loss in the study group

Total plasma homocysteine concentration (μmol/l)	Early RPL	Late RPL	t	p
	13,50±1,01	9,98±1,69	1.7879	>0.05

Patients who had ≥3 abortions showed a significantly higher total serum homocysteine concentration (15,76± 1,82 μmol/l) when compared to patients who had 2 spontaneous abortions (12,51±1,18 μmol/l) (p >0.05) (tab. 3).

Table 3

Total plasma homocysteine concentration according to the number of abortions in the study group

Total plasma homocysteine concentration (μmol/l)	2 spontaneous abortions	≥3 spontaneous abortions	t	p
	12,51±1,18	15,76±1,82	1.49783	>0.05

Hyperhomocyteinemia can cause obstetrical diseases that are connected with vascular disorders of pregnancy or the uteroplacental unit [8]. Gris et al. [5] reported an association between increased levels of homocysteine and a first early pregnancy loss. Kumar et al. [6] considered elevated homocysteine levels to be a risk factor for recurrent pregnancy loss.

In the current study, we found that the incidence of hyperhomocysteinemia was higher in women with unexplained primary early recurrent miscarriages.

Nutritional factors, particularly consumption and seric concentration of folate, vitamin B_{12} and B_6, seem to be the major parameters in homocysteine metabolization. Deficiencies, isolated or combined, of vitamins involved in the various pathways of homocysteine metabolism would be important markers for hyperhomocysteine [7].

An adequate intake of at least 400 μg of folate per day is difficult to maintain even with a balanced diet, and high-risk groups often find it impossible to meet these folate requirements. The bioavailability of dietary folates is 55 %. As the recommendation to eat a healthy diet has little or limited impact on elevated homocysteine levels, (folate)-fortified foods and/or vitamin supplements are rational and therefore recommended. [13]

Maternal hyperhomocysteinemia is related to birth defects, including neural tube defects (NTDs), orofacial clefts, clubfoot, and Down syndrome. Folic acid supplements in the periconceptual period and the first few weeks of pregnancy reduce the risk of NTDs [10,11]. For this reason, all women of childbearing age should have a folate intake of at least 400 μg/day [10,17].

Vitamin supplementation continues to be a recommendable option for prophylaxis of hyperhomocysteinemia. Dosages for prophylaxis are given in Figure 5 (low-dose supplementation: folic acid – 0.2 to 0.8 mg/day; vitamin B_{12} – 3 to 100 μg/day; vitamin B_6 – 2 to 25 mg/day). If this supplementation regimen lowers plasma homocysteine to <10 μmol/l within 4 weeks, repeated measurements of plasma homocysteine should be obtained first every 6 months and later on once a year. If response (plasma homocysteine reduction) is still inadequate, the dosage of folic acid should be increased to, say, 1 to 5 mg of folic acid per day (while supplementation with vitamin B_{12} and vitamin B_6 can be continued unchanged for some time). Repeated determinations of plasma homocysteine should be performed at 4-week intervals. [13]

There are biologically plausible reasons that increased total serum homocysteine may be related to adverse pregnancy outcomes. Increased total serum homocysteine may directly or indirectly cause endothelial dysfunction, impair neurolation, reduce microfilament synthesis, inhibit DNA methylation and alter gene expression, and reduce *S*-adenosylmethionine-dependent methylation reactions [10].

Regarding plasma levels of homocysteine, subjects were divided into two groups: group I – women with plasma homocysteine concentration < 10 μmol/l (n = 16) and group II – women with plasma homocysteine concentration > 10 μmol/l (n = 32).

In our study, we recommended a complex of B vitamin supplementation (folic acid – 0,4 mg/day; vitamin B_{12} – 3 to 100 μg/day; vitamin B_6 – 2 to 25 mg/day) for women with plasma homocysteine concentration < 10 μmol/l and a complex of B vitamin supplementation (folic acid:1 – 5 mg; vitamina B_{12}: 100 – 600 μg; vitamina B_6: 6 – 25 mg) for women with plasma homocysteine concentration > 10 μmol/l. Also, everybody was recommended to eat a diet rich in vitamins.

The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception. As a result, in the first group only 10 women (62,5%, 95% CI 38,78 – 86,22) from 16 women became pregnant. In the second group, 32 women (94,11%, 95% CI 48,01 – 140,21) became

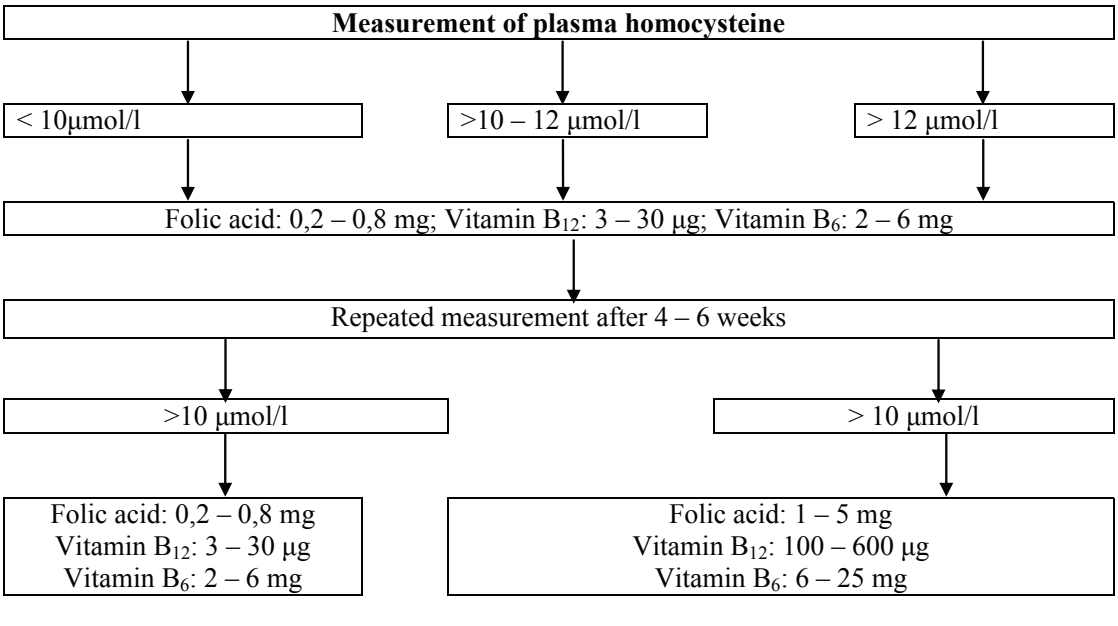

Fig. 5. Decision tree for diagnosis and prophylaxis/treatment of hyperhomocysteinemia [13].

pregnant, of which 2 women had a miscarriage in the first trimester of pregnancy. All the pregnant patients passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy. However, because of the small number of selected patients, our data should be confirmed by further studies based on larger population.

Conclusions

1. Our study provides data concerning the involvement of homocysteine in women with RPL without other causes of recurrent abortion. We found that the incidence of hyperhomocysteinemia was higher in women with unexplained primary early recurrent miscarriages.

2. The complex of B vitamin supplementation and, also, a diet rich in vitamins was recommended at least 2 to 3 months before conception.

3. In the current study, 40 women (80,0%, 95% CI 68,92 – 91,08) became pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.

4. Because of the small number of selected patients, our data should be confirmed by further studies based on larger population.

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