Coll. Antropol. 38 (2014) 4: 1153–1161
Original scientific paper

The Effect of Trombophilia on Pregnancy Outcome and IVF Success

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ABSTRACT

Thrombophilia is a group of inherited and acquired coagulation disorders often associated with an increased risk of thrombosis. Over the last decade, inherited thrombophilia is often referred as a possible cause of recurrent miscarriages and in vitro fertilisation (IVF) failure. However, many studies in this area still give conflicting results, so the goal of our study was to determine the effect of thrombophilia on pregnancy outcome and success of IVF. The study included 38 patients with proven gene mutation for thrombophilia and 53 patients without mutations. The studied parameters were age, duration of infertility, dose of gonadotropins, duration of stimulation, number of embryo transfer (ET), number of oocytes retrieved, the number of days to ET and the outcome in terms of delivery (full term or premature), ectopic pregnancy or abortion. There was no significant difference between two groups in the number of procedures performed, the number of twin pregnancies, abortions, twin miscarriages, ectopic pregnancies, births and the etiology of infertility. A statistically significant difference was found in the number of pregnancies (p=0.018) and in duration of infertility which was significantly longer in the group with thrombophilia (p<0.001). The number of abortions in homozygous PAI-1 was significantly more common (p=0.012). Procedures in natural cycle were significantly more frequent in group with thrombophila (p=0.011), so we recommend in patients with proven mutation first to start with procedures in the natural cycle, and only in case of failure to use the possibility of stimulating cycles. In conclusion, in patients on anticoagulant therapy a higher percentage of IVF failure has not been proven. Therefore, we strongly recommend the prophylactic use of low molecular weight heparin during pregnancy and screening for the most common mutations in our population, particularly in patients with a history of IVF failure and those with a long duration of infertility.

Key words: *inherited thrombophilia, IVF, low molecular weight heparin*

Introduction

Thrombophilia is a group of various hereditary and acquired coagulation disorders often associated with an increased risk of thrombosis. Various studies have shown that the success of *in vitro* fertilisation (IVF) in patients with thrombophilia is about 30%[1]. Several factors have been recognized to affect either success or failure rate of IVF/ET. Such factors include age, basal hormone levels, previously successful pregnancy, endometrial thickness, embryo grading and technique of ET[2]. Over the last decade, inherited thrombophilia is oftenly referred as a possible cause of recurrent miscarriages, IVF/ET failure, embryo implantation and placentation. Further additional researches are needed in order to prove certain connections, because so far, they give conflicting results[3–6]. Many studies have suggested that the reason for the increased IVF failure is hypercoagulability, manifested by thrombosis of maternal blood vessels of placental bed[3,5]. Hereditary thrombophilia is found in 1% of the general population, while the Factor V Leiden mutation, which is also the most common one found in about 5% of the Caucasian[7]. Other frequent mutations are ones in the gene for prothrombin G20210A, antithrombin, protein C and S deficiency, mutations in the methyltetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI-1) gene.

Consequently, screening and treatment of patients carriers of mutations is often used to improve obstetric outcomes[8]. That is why many recommend screening for thrombophilia in women before engaging in IVF treatment. Unfortunately, the efficacy of this form of interven-

Received for publication September 29, 2014

tion is still unclear. Therefore the goal of our study was to determine the effect of thrombophilia on pregnancy outcome and success of IVF.

Subjects and Methods

Subjects

The study was conducted between 2011 and 2013 at the Division of Human Reproduction and Gynecological Endocrinology, Department of Gynecology and Obstetrics, Medical School University of Zagreb, Croatia. The study included 91 patients involved in the IVF procedure.

Indications for IVF were anovulation, unexplained infertility and male or female factor of infertility. The inclusion criterion was infertility in duration of at least a year. The patients were divided into 2 groups. The first group (A) included 38 patients with mutations of the genes for thrombophilia (Table 1). The control group (B) comprised

TABLE 1
FREQUENCY OF CERTAIN MUTATIONS

		N	%
PAI-1	Homozygous	4	26.7%
	Heterozygous	11	73.3%
MTHFR	Homozygous	2	33.3%
	Heterozygous	4	66.7%
Double mutation	homozygous PAI-1+MTHFR	2	13.3%
	heterozygous PAI-1+MTHFR	10	66.7%
	homozygous PAI-1+heterozygous MTHFR	2	13.3%
	heterozygous PAI-1+homozygous MTHFR	1	6.7%
Triple mutation	homozygous PAI-1+MTHFR+FII	1	50.0%
	heterozygous PAI-1+MTHFR+FII	1	50.0%

PAI-1 – plasminogen activitor inhibitor, MTHFR – methyltetrahydrofolate reductase

TABLE 2
COMPARISON OF CATEGORICAL VARIABLES BETWEEN GROUPS WITH THROMBOPHILIA (A) AND WITHOUT THROMBOPHILIA (B): CHI-SQUARE TEST

		Groups				p-value
		Without thrombophilia (B)		Thrombophilia (A)		
		N	%	N	%	
Procedures	1	34	64.2%	16	42.1%	0.07
	2	15	28.3%	11	28.9%	
	3	2	3.8%	6	15.8%	
	4	1	1.9%	4	10.5%	
	5	1	1.9%	1	2.6%	
Pregnancy	0	29	54.7%	15	39.5%	0.018
	1	24	45.3%	18	47.4%	
	2	0	.0%	5	13.2%	
Twin pregnancy	0	50	94.3%	36	94.7%	0.935
	1	3	5.7%	2	5.3%	
Abortion	0	48	90.6%	31	81.6%	0.211
	1	5	9.4%	7	18.4%	
Twin abortion	0	52	98.1%	38	100.0%	0.395
	1	1	1.9%	0	.0%	
Ectopic pregnancy	0	52	98.1%	35	92.1%	0.168
	1	1	1.9%	3	7.9%	
Birth	0	35	66.0%	20	52.6%	0.197
	1	18	34.0%	18	47.4%	
Time of birth	Full term	17	94.4%	16	88.9%	0.546
	Premature	1	5.6%	2	11.1%	
Natural cycle	0	52	98.1%	29	76.3%	0.011
	1	1	1.9%	4	10.5%	
	2	0	.0%	4	10.5%	
	4	0	.0%	1	2.6%	
Cause of infertility	Male	17	32.1%	16	42.1%	0.608
	Female	12	22.6%	8	21.1%	
	Mix	10	18.9%	8	21.1%	
	Unknown	14	26.4%	6	15.8%	

TABLE 3

DIFFERENCES IN MEDIAN VALUES OF QUANTITATIVE VARIABLES BETWEEN GROUPS MANN-WHITNEY U TEST

	Groups	N	Min	Max	Percentiles			p
					25th	50th (Median)	75th	
Age	Without thrombophilia	53	25.00	43.00	30.00	34.00	37.00	0.215
	Thrombophilia	38	28.00	43.00	31.00	34.00	39.25	
Duration of infertility	Without thrombophilia	49	1.00	13.00	2.00	4.00	5.00	<0.001
	Thrombophilia	36	2.00	12.00	4.00	6.00	8.00	
Number of oocytes	Without thrombophilia	53	1.00	32.00	3.00	6.00	9.00	0.332
	Thrombophilia	38	1.00	24.00	3.00	6.50	11.25	
ET after X days	Without thrombophilia	53	3.00	17.00	3.00	5.00	6.00	0.087
	Thrombophilia	37	3.00	14.00	3.00	5.00	8.50	
Dose of gonadotropins	Without thrombophilia	53	825.00	15675.00	1425.00	2025.00	3050.00	0.449
	Thrombophilia	38	900.00	5175.00	1425.00	2512.50	3468.75	
Duration of stimulation	Without thrombophilia	53	8.00	58.00	9.50	11.00	23.00	0.398
	Thrombophilia	38	7.00	44.00	10.00	16.50	24.00	
Number of ET	Without thrombophilia	53	1.00	9.00	2.00	2.00	3.00	0.080
	Thrombophilia	38	1.00	7.00	2.00	2.00	4.00	

ET – embryo transfer

53 patients without mutations. Exclusion criteries were previous thromboembolic incidents. All 38 patients from the first group, in the case of a positive beta-human chorionic gonadotropin (βHCG) 14 days after IVF procedure, were treated with 0.4 mL (1750 IU) of low molecular weight heparin until the end of pregnancy. A total of 156 procedures were done, 77 in group A and 79 in group B. Out of 156 procedures, 17 of them were done in the natural cycle, 16 in group A and 1 in group B. In the remaining 139 procedures all patients received standardized protocol ofcontrolled ovarian stimulation using the combination of gonadotropin-releasing hormone(GnRH) agonists and gondotropins. ET was preformed after each procedure. Patients age in both groups was approximately equal, with a range from 28 to 43 years (interquartile range 31.00 to 39.25) in group A and 25 to 43 years (interquartile range 30.00 to 37.00) in group B.

Studied parameters were age, duration of infertility, dose of gonadotropins, duration of stimulation, number of ET, number of retrieved oocytes, number of days until the ET and the outcomes, in terms of delivery (full term or premature), ectopic pregnancy or abortion.

Statistical analysis

The results were statistically analyzed and presented as percentages and medians (25–75 interquartile range). Chi-square test, the Mann-Whitney U test and Kruskal-Wallis test were used. P-values less than or equal to 0.05 are considered statistically significant. Data analysis was performed in Statistica 10.0 (StatSoft, Inc., 2011, STATISTICA data analysis software system version 10), and Microsoft Office Excel 2003 program.

Results

The results of our study are presented in Table 2. There was no no significant difference between two groups in the number of procedures performed, twin pregnancies, number of abortions, number of twin miscarriages, ectopic pregnancies, number of births or etiology of infertility. A statistically significant difference was found in the number of pregnancies (p=0.018) and in the number of procedures performed in the natural cycle (p=0.011). In group A, there were a total of 23 pregnancies, 18 patients with 1 pregnancy (47.4%) and 5 patients with 2 pregnancies (13.2%), while in group B, there were a total of 24 pregnancies (45.3%).

The procedures in the natural cycle were significantly more frequent in group A. In the total of 16 procedures, 4 patients (10.5%) had one procedure in a natural cycle, 4 patients (10.5%) had two procedures in natural cycle and only 1 patient (2.6%) had four procedures in the natural cycle. Statistically significant differences in median values of quantitative variables between groups were found in the duration of infertility, which was significantly longer in thegroup with thrombophilia (A) (interquartile range 4–8) compared to group B (interquartile range 2–5) (p<0.001).

TABLE 4

DIFFERENCES IN CATEGORICAL VARIABLES IN A GROUP OF THROMBOPHILIC PATIENTS ACCORDING TO THE TYPE OF MUTATION

		Mutation								p-value
		PAI1		MTHFR		Double mutation		Triple mutation		
		N	%	N	%	N	%	N	%	
Procedures	1	6	40.0%	3	50.0%	4	57.1%	1	50.0%	0.771
	2	4	26.7%	3	50.0%	2	28.6%	0	.0%	
	3	3	20.0%	0	.0%	0	.0%	1	50.0%	
	4	1	6.7%	0	.0%	1	14.3%	0	.0%	
	5	1	6.7%	0	.0%	0	.0%	0	.0%	
Pregnancy	0	7	46.7%	2	33.3%	3	42.9%	1	50.0%	0.34
	1	7	46.7%	2	33.3%	4	57.1%	0	.0%	
	2	1	6.7%	2	33.3%	0	.0%	1	50.0%	
Twin pregnancy	0	15	100.0%	5	83.3%	7	100.0%	2	100.0%	0.247
	1	0	.0%	1	16.7%	0	.0%	0	.0%	
Abortion	0	13	86.7%	4	66.7%	6	85.7%	1	50.0%	0.503
	1	2	13.3%	2	33.3%	1	14.3%	1	50.0%	
Twin abortion	0	15	100.0%	6	100.0%	7	100.0%	2	100.0%	–
Ectopic pregnancy	0	14	93.3%	6	100.0%	6	85.7%	1	50.0%	0.208
	1	1	6.7%	0	.0%	1	14.3%	1	50.0%	
Birth	0	9	60.0%	2	33.3%	5	71.4%	2	100.0%	0.322
	1	6	40.0%	4	66.7%	2	28.6%	0	.0%	
Time of birth	Full term	6	100.0%	3	75.0%	2	100.0%	0	.0%	0.336
	Premature	0	.0%	1	25.0%	0	.0%	0	.0%	
Natural cycle	0	11	73.3%	5	83.3%	6	85.7%	2	100.0%	0.93
	1	2	13.3%	1	16.7%	0	.0%	0	.0%	
	2	1	6.7%	0	.0%	1	14.3%	0	.0%	
	4	1	6.7%	0	.0%	0	.0%	0	.0%	
Cause of infertility	Male	8	53.3%	2	33.3%	4	57.1%	0	.0%	0.15
	Female	1	6.7%	2	33.3%	1	14.3%	2	100.0%	
	Mix	4	26.7%	1	16.7%	0	.0%	0	.0%	
	Unknown	2	13.3%	1	16.7%	2	28.6%	0	.0%	

PAI-1 – plasminogen activitor inhibitor, MTHFR – methyltetrahydrofolate reductase

Comparing the age of the patients, number of retrieved oocytes, dose of used gonadotropins, duration of stimulation, number of days until ET and number of ET, we did not find significant differences (Table 3). Considering the type of mutation, in group A there were no significant differences in categorical variables (Table 4).

Comparing the quantitative variables in group A, regarding the type of mutation, we did not find significant differences (Table 5). Due to the small number of MTHFR, certain double and triple mutation the analysis was conducted only with PAI-1 mutation. The comparison of categorical variables between PAI-1 homozygous and heterozygous, demonstrated a statistically significant difference in the number of abortions. In PAI-1 homozygous abortion was significantly more common (p=0.012) (Table 6). In the 38 patients in group A, 15 of them were positive for the PAI-1 mutation, 4 homozygotes (26.7%) and 11 heterozygotes (73.3%). In this group of patients 2 miscarriages (50%) were recorded, both PAI-1 homozygotes.

TABLE 5

DIFFERENCES IN QUANTITATIVE VARIABLES IN A GROUP OF THROMBOPHILIC PATIENTS ACCORDING TO THE TYPE OF MUTATION: THE KRUSKAL-WALLIS TEST

	Mutation	N	Min	Max	Percentiles			p
					25th	50th (Median)	75th	
Age	PAI1	15	30	43	33.00	35.00	40.00	0.609
	MTHFR	6	28	40	28.75	33.50	40.00	
	Double mutation	7	30	42	31.00	34.00	40.00	
	Triple mutation	2	33	34	24.75	33.50	25.75	
Duration of infertility	PAI1	14	2	12	4.00	8.00	10.00	0.124
	MTHFR	6	3	6	3.75	4.50	6.00	
	Double mutation	7	3	12	4.00	6.00	7.00	
	Triple mutation	2	3	4	2.25	3.50	3.25	
Number of oocytes	PAI1	15	2	24	3.00	4.00	11.00	0.523
	MTHFR	6	6	20	6.00	8.00	13.25	
	Double mutation	7	1	21	3.00	9.00	10.00	
	Triple mutation	2	6	17	4.50	11.50	13.00	
ET after X days	PAI1	15	3	13	3.00	5.00	8.00	0.980
	MTHFR	6	5	8	5.00	5.00	6.50	
	Double mutation	7	3	12	3.00	5.00	9.00	
	Triple mutation	2	3	13	2.25	8.00	10.00	
Dose of gonadotropins	PAI1	15	1350.0	5175.0	1800.000	2250.000	3450.000	0.519
	MTHFR	6	1150.0	3975.0	1356.250	2156.250	3468.750	
	Double mutation	7	900.0	3525.0	950.000	1575.000	2925.000	
	Triple mutation	2	1200.0	4125.0	900.000	2662.500	3094.000	
Duration of stimulation	PAI1	15	9	34	10.00	16.00	20.00	0.645
	MTHFR	6	10	44	10.00	19.50	35.00	
	Double mutation	7	7	24	9.00	11.00	19.00	
	Triple mutation	2	9	35	6.75	22.00	26.50	
Number of ET	PAI1	15	1	6	2.00	2.00	4.00	0.856
	MTHFR	6	1	4	1.75	3.00	4.00	
	Dvostruka mutacija	7	1	7	1.00	2.00	3.00	
	Trostruka mutacija	2	2	6	1.50	4.00	4.75	

PAI-1 – plasminogen activitor inhibitor, MTHFR – methyltetrahydrofolate reductase, ET – embryo transfer

Comparing the quantitative variables in PAI-1 homozygotes and heterozygotes we did not find statistically significant differences (Table 7). No thrombotic complications occured during IVF procedure or pregnancy in group A.

Discussion and Conclusion

This study showed that the presence of thrombophilia (PAI-1, MTHFR, double and triple mutations) does not increase the risk of IVF failure. In 38 patients from group A, 28 pregnancies were recorded, and in 53 patients from group B, 24 pregnancies, reaching statistical significance of p=0.018. In patients with thrombophilia we recorded a slightly higher number of abortions and ectopic pregnancies, however, they have not reached statistical significance. In group A, there were 47.4% live births, and 34% in group B, but statistically significant differences was not found. Our research has demonstrated significantly longer duration of infertility among women with thrombophilia.

TABLE 6

DIFFERENCES IN CATEGORICAL VARIABLES BETWEEN HOMOZYGOUS AND HETEROZYGOUS PAI-1

		PAI-1				p-value
		Homozygous		Heterozygous		
		N	%	N	%	
Procedures	1	2	50.0%	4	36.4%	0.311
	2	0	.0%	4	36.4%	
	3	2	50.0%	1	9.1%	
	4	0	.0%	1	9.1%	
	5	0	.0%	1	9.1%	
pregnancy	0	2	50.0%	5	45.5%	0.191
	1	1	25.0%	6	54.5%	
	2	1	25.0%	0	.0%	
Twin pregnancy	0	4	100.0%	11	100.0%	–
Abortion	0	2	50.0%	11	100.0%	0.012
	1	2	50.0%	0	.0%	
Twin abortion	0	4	100.0%	11	100.0%	–
Ectopic pregnancy	0	3	75.0%	11	100.0%	0.086
	1	1	25.0%	0	.0%	
Birth	0	4	100.0%	5	45.5%	0.057
	1	0	.0%	6	54.5%	
Time of birth	Full term	0	.0%	6	100.0%	–
	Premature	0	.0%	0	.0%	
Natural cycle	0	4	100.0%	7	63.6%	0.576
	1	0	.0%	2	18.2%	
	2	0	.0%	1	9.1%	
	4	0	.0%	1	9.1%	
Cause of infertility	Male	2	50.0%	6	54.5%	0.321
	Female	1	25.0%	0	.0%	
	Mix	1	25.0%	3	27.3%	
	Unknown	0	.0%	2	18.2%	

PAI-1 – plasminogen activitor inhibitor

Despite numerous studies in this area, definitive answers are not yet obtained. Qublanet al. have proved the existence of association of thrombophilia and implantation failure[9]. In their case-control studies 90 and 45 patients with recurrent IVF failures were included, and 90 (100), and 44 patients in the control group. Qublan et al. have proved the existence of more than three gene mutations among 10 investigated genes, in 74% of women with implantation failure compared to 20% of patients in the control group (p=0.0004) [9]. They concluded that hereditary thrombophilia is associated with implantation failure. The study results of Azem et al. (2004) showed that 42.9% of patients with 4 or more failed IVF cycles are positive for thrombophilia mutations, compared with 18.2% in the control group, and that everything points that inherited thrombophilia plays a role in the etiology of recurrent IVF failures, particularly in the subgroup with unexplained infertility[5].

The results of our research, on the impact of thrombophilia on implantation, coincide with a number of studies[10,11]. Meta-analysis of Di Nisio et al. (2011) included a

TABLE 7

DIFFERNCES IN QUANTITATIVE VARIABLES IN THE GROUP OF THROMBOPHILIC PATIENTS DUE TO THE PRESENCE OF PAI-1 MUTATION: MANN-WHITNEY U TEST

	PAI-1	N	Min	Max	Percentiles			p
					25th	50th (Median)	75th	
Age	Homozygous	4	31	41	32.00	37.00	40.50	0.851
	Heterozygous	11	30	43	33.00	35.00	40.00	
Duration of infertility	Homozygous	4	2	10	2.25	6.50	10.00	0.733
	Heterozygous	10	4	12	5.50	8.00	9.25	
Number of oocytes	Homozygous	4	3	11	3.75	7.50	10.50	0.571
	Heterozygous	11	2	24	3.00	3.00	12.00	
ET after X days	Homozygous	4	3	13	3.00	6.00	12.00	0.753
	Heterozygous	11	3	9	3.00	5.00	6.00	
Dose of gonadotropins	Homozygous	4	1350.0	5175.0	1518.750	2925.000	4837.500	0.661
	Heterozygous	11	1350.0	3900.0	1800.000	2250.000	3300.000	
Duration of stimulation	Homozygous	4	10	34	10.00	19.00	32.50	0.661
	Heterozygous	11	9	29	10.00	16.00	19.00	
Number of ET	Homozygous	4	2	6	2.00	3.50	5.75	0.343
	Heterozygous	11	1	6	2.00	2.00	3.00	

PAI-1 – plasminogen activitor inhibitor, ET – embryo transfer

total of 33 studies with 6092 patients and showed a higher failure rate in patients with Factor V Leiden mutation while other mutations, including MTHFR are proven as risk factors for adverse outcome[10]. Results of Roque et al. (2004) show that the presence of thrombophilia (Factor V Leiden and prothrombin) is not associated with pregnancy loss of less than 10 weeks, on the other hand, a number of meta-analyzes confirm the correlation especially in Factor V Leiden mutation with pregnancy loss[12,13]. But before the final interpretation of our results we should consider the fact that all of our patients have been on the low molecular weight heparin, in case of a positive βHCG. Question is whether low molecular weight heparin is the one that actually canceled the possible impact of thrombophilia on implantation and whether we should introduce heparin prophylaxis in all patients with thrombophilia?

There are studies that negate the need for screening for thrombophilia and which do not justify prophylactic administration of heparin, but there are studies that suggest just the opposite, supporting screening and administration of heparin. Research of Brenner et al. (2000) showed an increase in successfully completed pregnancies with the heparin treatment, from 20% in the untreated control group to 75% in the treated group[4,14–16]. There are studies that compare the efficacy of taking the combination of low molecular weight heparin with aspirin or aspirin intake only. Some studies provide very significant preferences in the use of heparin prophylaxis while others refute these results, arguing that in asymptomatic patients with thrombophilia is possible to achieve good outcomes without treatment[17,18]. Laskin et al. (2009) failed to prove a significant advantage of the combination of heparin and aspirin compared to aspirin use alone[19]. While there is no evidence of adverse affects of aspirin or heparin, most doctors, if prophylaxis is needed, recommend administration of heparin[17]. To confirm the prophylactic administration of heparin a number of randomized multicentric studies, with a large number of patients with different types of mutations, are required. Also, it is important to note that it is very difficult to give the final answer on the impact of thrombophilia on the outcome of IVF, because all researches generally consider only mother's thrombophilia, while possible mutations in the embryo or fetus and father are not taken into account as important factor that may also have an impact on the outcome of IVF.

In our study,mutations in the gene for PAI-1 were confirmed in 15 patients (39.47%). 11 of 15 patients were heterozygotes and 4 homozygotes for the same mutation. Urokinase plasminogen activator and its receptor and PAI-1 are believed to control proteolysis and transformation of maternal tissue during the invasion of the trophoblast[20,21]. PAI-1 levels were associated with a polymorphism of genes. The most common are insertion/deletion of guanine, 675 bp upstream of the translation start site. It has been shown that homozygosity for the deletion genotype 4G/4G is associated with higher concentrations of PAI-1 than in genotype 5G/5G, and consequently with reduced fibrinolytic activity[22,23]. Buchholz[24] et al. (2003),

have shown that the rate of abortions, and significantly higher relative risk of pregnancy loss are increased in this group of patients. Apart from mutations of PAI-1, Dossenbach-Glaninger et al. (2003) have shown that polymorphism of the coagulation factor XIII (FXIII) Val34Leu and 4G/5G PAI-1 interfere with crosslinking of fibrin and fibrinolysis and can lead to early pregnancy loss[25].

Our results showed significantly more frequent number of abortions in homozygous PAI-1, actually, none of those pregnancies resulted in childbirth. So far in the literature results related with the role of PAI-1 mutations show conflicting results. Before beginning our study all patients had their mutations confirmed, but since the mutation of PAI-1 resulted as the most common one in our study population, it would make sense to test all patients with long duration of infertility before conducting IVF or patients with recurrent IVF failures for the presence of these mutations. A significantly smaller number of patients, to be precise 6 (15.78%) were carriers of the MTHFR mutation, 2 homozygotes and 4 heterozygotes.

In most studies the incidence of MTHFR mutation was between 18.8 to 22.2%[4,5,9]. MTHFR is an enzyme that catalyzes the reduction of 5–10 methyltetrahydrofolate and allows re-methylation of homocysteine to methionine. MTHFR mutations can lead to hyperhomocysteinemia.

Over the past 20 years, many studies cited MTHFR mutation as a risk factor for thrombosis and pregnancy loss, while others refuted that connection[26–29]. The exact mode of development of complications in pregnancy is not known, although the hypothesis is that hyperhomocysteinemia leads to endothelial damage, resulting in venous thromboembolism and placental insufficiency[30]. However, other studies refute this assumption. When comparing the concentration of homocysteine in patients with and without a mutation of MTHFR, no significant difference is found. Suggesting that fetal losses are not associated with homocysteine levels, and very likely there are other mechanisms[31].

The analysis of our data did not detect a difference in categorical variables between individual mutations in the study. Due to the small number of patients, analysis of differences between homozygous and heterozygous MTHFR was not preformed. Similarly homocysteine levels were not measured, since all patients were on treatment with folic acid. Possible reasons for different results obtained are relatively small number of patients in our study, and the inability to detect significant differences that could be applied to most patients, in only 6 proven mutation, and for confirmation it is necessary to do the research on a larger number of patients.

From a total of 38 patients from group A, 15 of them (39.47%) had a confirmed double mutation. The most common form of double mutation (66.7%) was heterozygous combination of PAI-1 with heterozygous MTHFR, and the triple mutation was detected in only 2 patients. In our study, the existence of double or triple mutation has not been proven as a risk factor for pregnancy loss, however it should be noted that the number of patients included in study was relatively small. Coulam et al. (2006) showed that in the group of patients with recurrent implantation failure is significantly greater number of mutated genes 74% compared to the control group, 20%[6]. So far there were not any researches to determine whether there is greater risk of spontaneous abortion, pregnancy complications or IVF failure in patients with double or triple mutations compared to patients with evidence of only one mutation. The answer to that question still remains unclear.

Another statistically significant difference was found in this study, and that is the conduction of IVF procedure in a natural cycle (P=0.011) in the group positive for thrombophilia. In the group positive for thrombophilia we found greater number of pregnancies, so it could suggest significantly better IVF procedure outcome in the natural cycle in those patients. In patients with proven mutation, it is recommended to start first with procedures in the natural cycle, and only in case of failure to use the possibility of stimulation of cycles. However, for definitive proof of this results, further research is needed, especially using natural cycle in IVF procedures in a significant number of patients. In conclusion, in patients on heparin therapy a higher percentage of failed IVF procedures and pregnancies has not been proven. Therefore, we strongly recommend the prophylactic use of low molecular weight heparin during pregnancy and screening for the most common mutations in our population, particularly in patients with a history of failed IVF and those with a long duration of infertility.

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UTJECAJ TROMBOFILIJE NA ISHOD TRUDNOĆE I IVF POSTUPAKA

S A Ž E T A K

Trombofilija je skupina nasljednih i stečenih poremećaja koagulacije često povezanih s povećanom sklonošću trombozi. Tijekom posljednjeg desetljeća, nasljedna trombofilija često se spominje kao mogući uzrok ponavljajućih pobačaja i neuspjelih in vitro fertilizacija (IVF). Međutim, mnoge studije u tom području još uvijek daju proturječne rezultate, tako da je cilj našeg istraživanja bio utvrditi utjecaj trombofilije na ishod trudnoće i uspjeh IVF-a. U istraživanju je sudjelovalo 38 pacijentica s dokazanom mutacijom gena za trombofiliju i 53 pacijenatice bez mutacije. Istraživani parametri bili su dob, trajanje neplodnosti, doza gonadotropina, trajanje stimulacije, broj embrio transfera (ET), broj oocita, broj dana do ET i ishod svakog postupka u vidu poroda (terminski ili prijevremeni), ekstrauterine trudnoće ili pobačaja. Između skupine A i skupine B nije dokazana statistički značajna razlika u broju provedenih postupaka, blizanačkih trudnoća, broju pobačaja, broju blizanačkih pobačaja, ekstrauterinih trudnoća, broju poroda ili etiologiji neplodnosti. Statistički značajna razlika pronađena je u broju trudnoća ($p=0,018$) i u trajanju neplodnosti koja je značajno dulja u skupini s trombofilijom ($p<0.001$). Broj pobačaja u homozigota PAI-1 je bio značajno češći ($p=0,012$). Postupci u prirodnom ciklusu su bili znatno češći u skupini s trombofilijom ($p=0,011$), stoga preporučujemo kod bolesnika s dokazanom mutacijom prvo započeti s postupcima u prirodnom ciklusu, a tek u slučaju neuspjeha koristiti mogućnost stimulacije ciklusa. U zaključku, u pacijentica na terapiji heparinom nije dokazan veći postotak neuspjelih IVF postupaka i trudnoća. Stoga svakako preporučujemo profilaktičku primjenu niskomolekularnog heparina za vrijeme trudnoće te probir na najčešće mutacije u našoj populaciji, osobito u pacijentica s povijesti neuspjelih IVF postupaka i u onih s dugim trajanjem neplodnosti.