Original Paper

Therapeutic Options in Pregnant Women with Thrombophilia Depending on the Genetic Mutations- a Prospective Study

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ABSTRACT: The aim is to evaluate the effects of anticoagulant therapy (Enoxaparine) compared to antiaggregant therapy (Aspirin) on pregnancy outcome depending on the mutational status of clotting factors. The study was conducted prospectively over a 2 years period and included 110 pregnant women, of whom 80 patients diagnosed with hereditary thrombophilia and 30 healthy pregnant women, representing the control group. After careful observation, we concluded a positive influence of Enoxaparine on pregnancy outcome in pregnant women homozygous carriers for factor V Leiden gene mutation and compound heterozygous for Factor V Leiden and Prothrombin *G20210A* gene mutation. The study did not find significant positive results in women with heterozygous Factor V Leiden and *G20210A* prothrombin gene mutation who received anticoagulant therapy as well as pregnant women who received low-dose Aspirin compared with controls.

KEYWORDS: *Thrombophilia, Clexane, Aspirin, pregnancy outcome*

Introduction

Hereditary thrombophilia is a relatively common condition in which blood has an increased tendency to develop clots due to genetic mutations involved in the synthesis of clotting factors [1].

The importance of this genetic disorder among pregnant women is based on their potential role to develop venous thromboembolism and maternal and fetal complications such as preeclampsia (PE), intrauterine growth restriction (IUGR), antepartum fetal death, premature detachment of placenta, early or late spontaneous abortions, prematurity [2,3,4].

Procoagulant status may be more or less important depending on the affected genes, thus, the homozygous status, in particular Factor V Leiden gene mutation (FVL) and Prothrombin *G20210A* gene mutation (PII), or compound heterozigot FVL/PII and also antithrombin III deficiency, presents the greatest risk of developing venous thromboembolism (VTE) [5,6,7,8,9,10]. The therapeutic protocol depends on the gene mutational status and on the other hand on family or personal history of VTE or obstetric complications (recurrent pregnancy loss in second and third trimester, more than 2 pregnancy losses in first trimester >10 weeks but <15 weeks, antepartum fetal death, placental abruption or severe intrauterine growth restriction, severe unexplained preeclampsia, family history of thrombophilia or thrombotic disease under 40 years of age-stroke, acute myocardial infarction without a known cause). According to guidelines based on the type of thrombophilia they are divided in-increased risk (Antithrombin deficiency, homozygous Factor V Leiden mutation, homozygous Prothrombin gene mutation, compound Heterozygous Factor V Leiden and prothrombin gene mutations) and the therapeutic option is to use antepartum and postpartum anticoagulant prophylaxis in previous VTE or family history, and in no previous VTE only postpartum-prophylaxis for 6 weeks [11]. In Low Risk Thrombophilia (heterozygous Factor V Leiden mutation, heterozygous Prothrombin gene mutation, Protein S or C deficiency) antepartum and postpartum prophylaxis is indicated only in personal history of 2 or more VTE events [12,13,14]. Guidelines also recommend the use of low-dose Aspirin (75mg) in women at high or moderate risk of preeclampsia.

The aim is to establish clear indications and efficacy of anticoagulant treatment in patients with thrombophilia in order to limit the abuse of these drugs and at the same time a review of all therapeutic options used at pregnant women with thrombophilia according to National and International Guidelines for Obstetrics and Gynecology.

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Material and method

We conducted a prospective, comparative study of 110 patients-divided into two groups-one consisting of 80 multiparous women diagnosed with hereditary thrombophilia and a personal history of obstetric complications, a personal and family history of thrombosis, admitted in the Clinical Department of Hematology and Obstetrics and Gynecology at Filantropia City Hospital of Craiova, and Clinical Department of Obstetrics and Gynecology at Emergency County Hospital of Craiova and the other group of 30 healthy multiparous women without a personal or family history of thrombosis representing the control group, for a period of two years between January 2016 and December 2017. Patients were included in the study at the time of conception and up to 6 weeks after birth (gestational age being calculated based on the exact date of the last menstrual period). According to European and International Guidelines, we divided the 80 pregnant women into high risk and low risk trophophilia. Due to family and personal history of thrombosis, both high-risk and low-risk trophophilia followed an anticoagulant treatment with low molecular weight heparin (Enoxaparin) in prophylactic dose of 40mg every 12 hours, for 11-14 weeks of gestation until 6 weeks after birth. Pregnant women carriers for A1298C or C677T Methylenetetrahydrofolate reductase (MTHFR) or tissue plasminogen activator inhibitor (PAI) gene mutations and history of thrombosis and perinatal complications especially preeclampsia were subjected to low-dose Aspirin 75mg at 12 h from 11-14 weeks of gestation until birth.

This study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova and all the women gave their written consent for participation and also received the study information form.

For the statistical interpretation we used the SPSS 20 (Statistical Package for Social Science) software. The fundamental indicators for the descriptive analysis were: arithmetic mean, median, modal value and dispersion indicators (variability)-standard deviation, and for the prediction (regression), 95% Confidence Interval, Crosstabulation, Relative risk and Odds ratio, but also for qualitative and quantitative data analysis, Pearson test, Fisher test, Chi square test. It was considered an important statistical significance when P value was below 0.05 limits.

Results

The mean age of women in the study group (±standard deviation) is 28.75, while in the control group is 27.60, indicating two homogeneous groups. (Tab.1)

Table 1. Distribution of patients by age

	N	Min	Max	Mean	Std. deviation	Variace	95% CI
Study group	80	22	40	28.75	4.654	21.658	[27.71-29.79]
Control group	30	20	34	27.60	4.344	18.869	[25.98-29.22]

The patients in the study group were divided according to the chosen therapy: anticoagulant therapy group using Low molecular weight heparins-Enoxaparin (Clexane) in prophylactic dose 40mg every 12 hours, consisting of 46 patients and the group treated with low dose Aspirin 75mg at 12 hours, consisting of 34 pregnant women and the control group consisting in 30 healthy pregnant women (Fig.1).

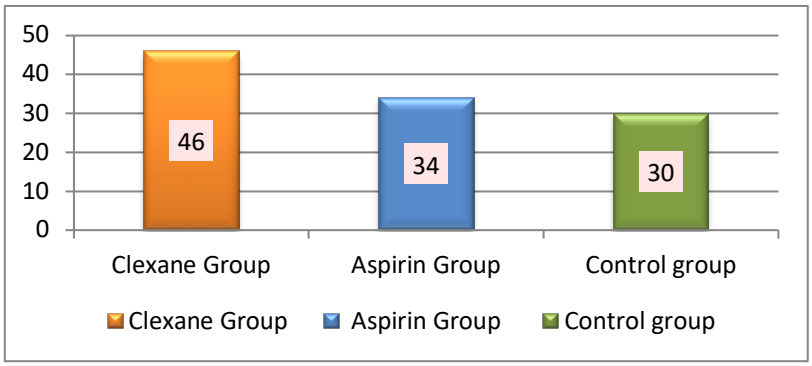

Fig.1. Distribution of pregnant women according to the chosen therapy

For clearer results, patients in the Clexane group were subdivided according to the mutation status of genes involved in coagulation factor synthesis in pregnant women with high risk thrombophilia 14.8% and with low risk thrombophilia 49.4% (Fig.2).

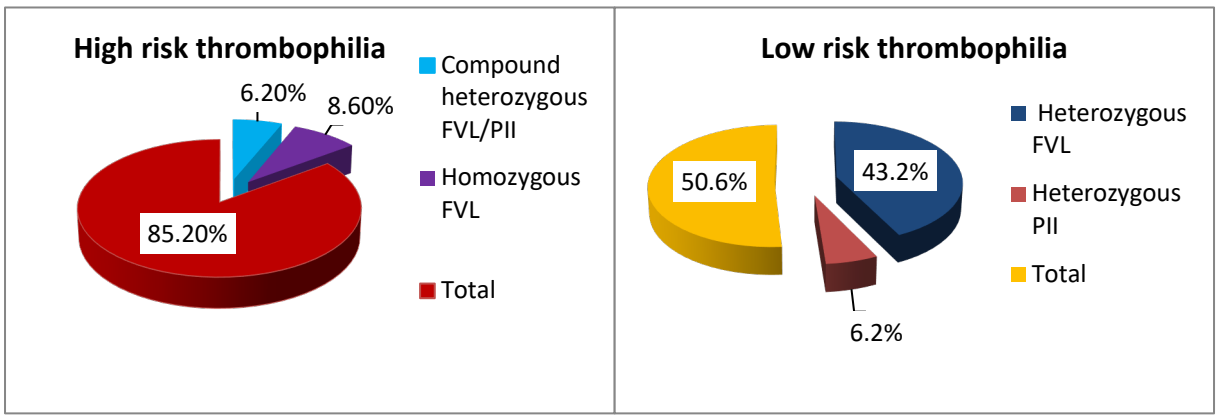

Fig.2. Distribution of patients depending on coagulation factors gene mutations

The likelihood that pregnant women undergoing Clexane treatment will develop obstetric complications depends on the mutagenic status of genes encoding clotting factors. The relative risk (RR) is the probability of developing a disease in the exposed group divided by the risk of carrying the disease in the unexposed group. In our case, patients carrying the FVL mutation (46 patients) had a 3.382 (CI 95%, 0.698-1.6401) fold higher risk of developing IUGR (3 cases)-compared with patients without FVL (34 patients) and 1.353 (CI 95%, 0.201-9.129) of developing preeclampsia (2 cases) (Table 2 and Table 3)

Table 2. The estimated risk (relative risk and odss ratio) of developing intrauterine growth restriction (IUGR) in patients carrying for factor V Leiden (FVL)

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for FVL (0/1)	0.264	0.048	1.451
Relative risk for cohort IUGR=negative FVL	0.892	0.766	1.039
Relative risk for cohort IUGR=positive FVL	3.382	0.698	16.401
Number of Valid Cases	80		

Table 3. The estimated risk (relative risk and odds ratio) of developing Preeclampsia (PE) in patients carrying for factor V Leiden (FVL)

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for FVL (0 / 1)	0.727	0.097	5.440
Relative risk for cohort PE=negative FVL	0.984	0.887	1.092
Relative risk for cohort PE=positive FVL	1.353	0.201	9.129
Number of Valid Cases	80		

In the case of treatment administration, the association between it and preeclampsia or IUGR does not reveal any statistical significance with P=0.7 and P=0.1 respectively, primarily due to the low number of cases that developed these complications due to the favorable response to therapy in some cases (Table 4 and Table 5).

It is important to underline that based on the mutagenic status; only patients with high risk thrombophilia have presented a spectacular response to anticoagulation therapy, so no patient in this group developed adverse pregnancy outcomes.

Table 4. The association between intrauterine growth restriction (IUGR) and Clexane treatment

		IUGR	Clexane
IUGR	Pearson Correlation	1	-,181
	Sig. (2-tailed)		,108
	N	80	80
Clexane	Pearson Correlation	-,181	1
	Sig. (2-tailed)	,108	
	N	80	80

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Table 5. The association between Preeclampsia (PE) and Clexane treatment

		Clexane	PE
Clexane	Pearson Correlation	1	-,035
	Sig. (2-tailed)		,759
	N	80	80
PE	Pearson Correlation	-,035	1
	Sig. (2-tailed)	,759	
	N	80	80

Regarding the group of patients with low-risk thrombophilia despite administration of Clexane therapy there were 6 cases who developed maternal and fetal complications (3 cases developed IUGR, 2 cases PE and 1 patient whose child was small for gestational age) suggesting the possibility of another cause of thrombosis and not hypercoagulability due to coagulation factors mutation (Tab.6). P value >0.05 without statistical significance, also due to the small number of cases that have developed perinatal complications making difficult any statistical correlation.

Table 6. Pregnancy outcome depends on high or low risk thrombophilia and Clexane treatment. OR-odds ratio; CI 95%-confidence interval

	High risk thrombophilia	Low risk thrombophilia	OR	CI 95%	P value
Gestational age <37 w	0	3 (6.5%)	0.714	[0.579- 0.881]	0.04
Gestational age >37 w	11 (23%)	32 (69%)	0.917	[0.831-1.012]	0.21
Birth weight <3000g	0	4 (8.6%)	1.375	[0.958-1.975]	0.001
Birth weight >3000g	11 (23%)	31 (67.3%)	7.556	[0.614-93.006]	0.07
Preeclampsia	0	2 (4.3%)	1.222	[0.925-1.615]	0.01

Despite the treatment with low doses of Aspirin, patients experienced complications associated with pregnancy, such as intrauterine growth restriction (5 out of 35 patients) and preeclampsia (2 out of 35 patients), without suggesting a possible influence of platelet antiaggregant therapy on pregnancy, similar results to those seen in patients with low risk thrombophilia but also in control group-3 patients developed IUGR and 2 patients PE.

By comparing the data from the two groups we can state that there is a similarity between Clexane-treated patients, Aspenter-treated patients and the control group due to similar number of pregnancy outcomes. Despite the administration of anticoagulant or antiaggregant therapy in patients with low-risk thrombophilia, there have been cases with perinatal complications, except for women with high risk thrombophilia, being the only ones who had a favorable response to Clexane therapy in the prophylactic dose.

Discussion

Focusing on the main purpose of the study, we can state that there is a clear correlation between anticoagulant treatment and the mutational status of coagulation factors in the development of perinatal complications, so the present study demonstrates a favorable influence of the pregnancy outcome in patients with high risk thrombophilia (homozygous carriers of FVl and *G20210A* PII, and double heterozigot FVL/PII), but without demonstrating the same in patients with low risk thrombophilia. At the same time, low-dose Aspirin therapy does not appear to influence the evolution of pregnancy in a significant way compared to the control group.

So far, there are many recommendations of anticoagulants use during pregnancy, but not all of them accurate and documented.

In the past 10 years, the use of anticoagulants in thrombophilia pregnant women is steadily rising, although some of these may cause serious maternal and fetal complications such as massive bleeding or teratogenicity, heparin-induced thrombocytopenia, osteoporosis, pain or haematomas at the injection site. Heparins are the most used anticoagulants due to the impossibility of crossing the placenta, starting with unfractionated heparin being the one that produces most complications, low molecular weight heparins-enoxaparin, dalteparin, tinzaparin or anti-factor Xa molecules-danaparoid [15,16].

Other anticoagulants such as antivitamin k are not recommended during pregnancy due to placental passage and teratogenic effects. Low molecular weight heparins (LMWH) are preferred to unfractionated heparins (UFH) in patients with venous thromboembolism (VTE) due to lower risk of developing maternal or fetal complications, and are used in both prophylactic and therapeutic doses depending on the history of thrombosis and the risk of developing VTE [17]. There is still insufficient data on anticoagulant dose adjustment during

pregnancy, raising the problem of dose adjustment by weight, if it is decided to choose the therapeutic dose of LMWH. However, the only possibility of adjusting the dose of anticoagulant is based on an anti-Xa level in a prophylactic or therapeutic dose between 0.3-0.6/0.6-1.0units/mL. No clear benefits have been reported when increasing the dose from 40mg to 80mg of Enoxaparin, with similar efficacy and safety of both doses with the birth of a living child in a proportion of 84% and 78% respectively [18].

A study of 109 pregnanct women with a previous history of VTE, revealed a recurrence rate of 10.9% during pregnancy and 3.7% outside of it (relative risk 3.5; 95% CI, 1.6-7.8) [19]. It appears that the risk of recurrence occurred more frequently in pregnant women with a previous episode of VTE without a known cause and antepartum/postpartum prophylaxis with LMWH is recommended.

In patients with thrombophilia, it seems that the relative risk of VTE recurrence varies depending on the genetic mutations of clotting factors, so patients with antithrombin deficiency present a risk of 9.5 (95% confidence interval; 1.6 to 31.9), those carrying heterozygous factor V Leiden gene mutation 6.4 (4.0 to 9.7), whereas the homozygous mutation had a significantly higher risk of 35.8, (0.4 to 137.8), values similar to the hetero/homozygote prothrombin gene mutation 5.1 and 21.2 respectively [20], similar results to those published by Simone et al. [21].

LMWH prophylaxis is suggested especially in pregnant women with previous obstetric events and carriers of FVL and/or PTG gene mutation, which seems to have a protective effect on the pregnancy loss (probability ratio, OR 0.52) [22]. In the case of pregnant patients without a previous episode of VTE their management with anticoagulation therapy remains controversial. Prophylaxis with LMWH should be given during pregnancy and post partum for 6 months at pregnant women with heterozygous mutation of FVL and a history of unprovoked VTE [13,24]; also antepartum in asymptomatic women who are homozygous for the FVL mutation or double heterozygote FVL/prothrombin gene mutation, but also in the case of a family history of VTE. In the absence of a family history, only postpartum prophylaxis with LMWH is suggested [13,24]. LMWH profilaxy should be reserved for high-risk populations with inherited thrombophilic mutations (double carriers of coagulation factors or history of thromboembolic events) [23].

Dabigatran-thrombin inhibitor and anti-Xa molecules-rivaroxaban, apixaban and edoxaban are not recommended during pregnancy or breastfeeding due to reproductive toxicity cited in some cases [25]. Low-dose acetylsalicylic acid (aspirin, 75mg/day) is recommended for the prevention of preeclampsia and its associated complications and should be initiated before the 20th week of gestation for women with moderate or high risk of preeclampsia or preeclampsia history of early onset or premature birth to less than 34 weeks of gestation or preeclampsia in more than one previous pregnancy [24,11].

Conclusions

This study provides information on the most thrombogenic mutations in coagulation factors and the indications and efficacy of anticoagulant therapy in pregnant women with thrombophilia. Our comparing data indicate a positive influence of anticoagulant therapy with Clexane on pregnancy outcome in prophylactic dose administered early since 11-14 week in patients with high risk thrombophilia, without obstetric complications or VTE. On the other hand LMWH administered in low-risk thrombophilia women appears to have small influence on the pregnancy outcome, with 3 cases developing IUGR and 2 cases developing preeclampsia. Also in the low-dose Aspirin group, we could not reveal an influence on the pregnancy outcome and of 33 patients, 7 cases (21%) developed obstetrical events, two of them PE and 5 RCIU similar results with control group. Anticoagulation therapy should only be reserved for patients with high risk thrombophilia.

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