PROCENA UČESTALOSTI TROMBOFILIJE KOD ISPITANICA SA GUBITKOM PLODA

ORIGINALNI RAD

ORIGINAL ARTICLE

EXAMINING THE PREVALENCE OF THROMBOPHILIA IN WOMEN WITH FETAL LOSS

Danijela Mandić[1,2]

[1] Univerzitet u Banjoj Luci, Medicinski fakultet, Banja Luka, Bosna i Hercegovina

[2] Univerzitetski klinički centar Republike Srpske, Klinika za unutrašnje bolesti, Odeljenje hematologije, Banja Luka, Bosna i Hercegovina

[1] *University of Banja Luka, Faculty of Medicine, Banja Luka, Bosnia and Herzegovina*

[2] *University Clinical Center of the Republic of Srpska, Clinic for Internal Medicine, Department of Hematology, Banja Luka, Bosnia and Herzegovina*

SAŽETAK

Uvod: Pored antifosfolipidnog sindroma (AFLS), nasledna trombofilija predstavlja jedan od najvažnijih hematoloških poremećaja koji mogu da dovedu do komplikacija trudnoće u vidu gubitaka ploda, zaostajanja u rastu i razvoju ploda (IUGR), preeklampsije i fetalne smrti. Učestalost spontanih pobačaja je do 20 % svih klinički prepoznatih trudnoća.

Cilj: Cilj rada je bio da se utvrdi učestalost urođene trombofilije kod ispitanica sa gubitkom ploda, analizira period trudnoće i godine života u kojima je došlo do gubitka ploda, kao i učestalost uspešnih trudnoća nakon profilaktičke primene heparina male molekulske težine (NMH).

Materijal i metode: Istraživanje je dizajnirano kao retrospektivna opservacijska studija u trajanju od 30 meseci na Klinici za ginekologiju i akušerstvo Univerzitetskog kliničkog centra Republike Srpske, kojom je obuhvaćeno 69 ispitanica sa jednim ili više gubitaka ploda u drugom i trećem trimestru trudnoće, ili dva i više ponavljanih gubitaka ploda u prvom trimestru trudnoće. Kod svih ispitanica su urađeni testovi za dokazivanja prisustva trombofilije.

Rezultati: Prosečna starost ispitanica je bila 30,7 godina, sa 167 prethodno neuspešnih trudnoća. Trombofilija je dokazana kod ukupno 40 ispitanica (58%). Rezultati su pokazali da su se kombinovane trombofilije, uključujući i polimorfizme, javljale s najvećom učestalosti od 47,5% (n=19). U grupi ispitanica sa trombofilijom primenjen je NMH kod 22 ispitanice uz uspešan ishod trudnoće kod 19 ispitanica.

Zaključak: Na osnovu sprovedenog istraživanja zaključuje se da kombinovane trombofilije, uključujući i kombinovane polimorfizme MTHFR i PAI-1, nose značajno veći rizik za gubitak trudnoće kod ispitanica svih starosnih grupa. Starost preko 35 godina nosi rizik za veće učestalosti spontanih pobačaja nezavisno od tipa nasledne trombofilije. Primena NMH značajno poboljšava ishode trudnoća kod ispitanica sa urođenom trombofilijom i prethodnim gubicima trudnoće.

Ključne riječi: trombofilija, pobačaj, heparin male molekulske težine

ABSTRACT

Introduction: In addition to antiphospholipid syndrome (APS), inherited thrombophilia is one of the most important hematologic disorders that can lead to pregnancy complications such as fetal loss, intrauterine growth restriction (IUGR), preeclampsia, and fetal death. The frequency of spontaneous abortions is up to 20% of all clinically recognized pregnancies.

Objective: The aim of this study was to determine the frequency of inherited thrombophilia in women with fetal loss, analyze the gestational period and age at which fetal loss occurred, and assess the frequency of successful pregnancies after prophylactic use of low molecular weight heparin (LMWH).

Material and methods: The study was designed as a 30-month retrospective observational study at the Clinic for Gynecology and Obstetrics of the University Clinical Center of the Republic of Srpska, involving 69 patients with one or more fetal losses in the second and third trimesters of pregnancy, or two or more repeated fetal losses in the first trimester of pregnancy. All patients underwent tests to detect the presence of thrombophilia.

Results: The average age of the patients was 30.7 years, and they had a total of 167 unsuccessful pregnancies. Thrombophilia was proven in a total of 40 patients (58%). The results showed that combined thrombophilia, including polymorphisms, occurred most frequently – 47.5% (n=19). In the group of patients with inherited thrombophilia, low molecular weight heparin (LMWH) was administered to 22 patients and it resulted in a successful pregnancy outcome in 19 patients.

Conclusion: Based on the conducted research, it can be concluded that combined thrombophilia, including combined polymorphisms MTHFR and PAI-1, carry a significantly higher risk of pregnancy loss in patients of all age groups. Age over 35 years is a risk factor for higher frequency of spontaneous abortions irrespective of the type of inherited thrombophilia. The use of LMWH significantly improves pregnancy outcomes in patients with inherited thrombophilia and previous pregnancy losses.

Keywords: trombophillia, fetal loss, low molecular weight heparin

Autor za korespondenciju:
Danijela Mandić
Univerzitetski klinički centar Republike Srpske
Ul. 12 beba bb, 78000 Banja Luka, Bosna i Hercegovina
Elektronska adresa: danijela.mandic@kc-bl.com

Corresponding author:
Danijela Mandić
University Clinical Center of the Republic of Srpska
12 beba Street, 78000 Banja Luka, Bosnia and Herzegovina
E-mail: danijela.mandic@kc-bl.com

Primljeno • *Received*: March 10, 2024; **Revidirano • *Revised*:** May 13, 2024; **Prihvaćeno • *Accepted*:** May 14, 2024; ***Online first*:** June 25, 2024

DOI: 10.5937/smclk5-49756

UVOD

Trombofilija je nasledan ili stečeni poremećaj hemostaze koji predisponira ili povećava rizik za nastanak tromboze i rekurentne tromboze [1,2]. Nasledne trombofilije se mogu klasifikovati u dve kategorije. Prvu kategoriju odlikuje nedostatak inhibitora koagulacije: antitrombina (AT), proteina C (PC) i proteina S (PS), a u drugu kategoriju se ubrajaju: rezistencija na aktivirani protein C (APC), Faktor V Leiden, mutacija gena za protrombin (F2), povećan nivo faktora FVIII i FIX, snižen FXII i disfibrinogenemije [3]. Nedostatak prirodnih inhibitora podrazumeva retke ali jake nasledne trombofilije. Mutacije u genima faktora Leiden i F2 čine do 70% dijagnostikovanih naslednih trombofilija i predstavljaju značajnije trombofilije [3]. Po trenutnim preporukama polimorfizam enzima metilen tetrahidrofolat-reduktaze (MTHFR) i inhibitor aktivatora plazminogena (PAI-1) se ne smatraju naslednim trombofilijama, iako su se ranije ova dva polimorfizma testirala u okviru laboratorijskih testova za trombofiliju [3-5].

Trudnoća predstavlja fiziološko hiperkoagulabilno stanje zbog promena u sistemu hemostaze tokom perioda trudnoće i postpartalno. Hiperkoagulabilnost je posledica promena u koagulacionom i fibrinolitičkom sistemu, a koje se manifestuje porastom faktora koagulacije (faktora II, VII, VIII, X, XII) i fibrinogena, odnosno pojačanim stvaranjem fibrina i smanjenom aktivnošću fibrinolitičkog sistema [6]. Novija istraživanja su pokazala da trombofilija može da potencira negativno dejstvo ovih promena, i pored izazivanja maternalnih komplikacija može da uzrokuje i probleme vezane za samu trudnoću: gubitak ploda, zaostajanje u rastu i razvoju ploda (IUGR), preeklampsiju i fetalnu smrt [7,8].

Ponovljeni gubitak trudnoće kod žena reproduktivne dobi, pored emotivnog problema predstavlja i socijalno ekonomski problem društva [9,10]. Smatra se da su uzroci gubitka ploda u ranoj fazi trudnoće anatomske abnormalnosti materice, kariotipske abnormalnosti i defekt u produkciji progesterona, dok su najvažniji hematološki poremećaji koji mogu da dovedu do gubitka ploda prisustvo antifosfolipidnih antitela i urođene trombofilije [11]. Uspešan ishod trudnoće zavisi od razvoja adekvatne placentalne cirkulacije. Placenta ima dvostruku cirkulaciju: maternalnu u interviloznim prostorima i fetalnu, koju čine krvni sudovi horda i horionskih resica. Uspešnost trudnoće zavisi od mehanizama koji preveniraju koagulaciju u viloznom i fetalnom krvotoku [6]. Ukoliko postoji hiperkoagulabilnost od strane majke, dolazi do smanjenog protoka u interviloznim prostorima, uz visok rizik za nastanak i odlaganje fibrinskih depozita u placenti koje mogu da dovedu da infarkta placente. Ukoliko je zahvaćeno više od 5% placentarne mase povećan je perinatalni mor-

INTRODUCTION

Thrombophilia is a hereditary or acquired disorder of hemostasis which predisposes or increases the risk of thrombosis or recurrent thrombosis [1,2]. There are two types of hereditary thrombophilia. The first category is characterized by a lack of coagulation inhibitors (i.e., antithrombin (AT), protein C (PC) and protein S (PS)), whereas the second category includes: activated protein C resistance (APCR), Factor V Leiden, prothrombin (F2) gene mutation, elevated levels of both FVIII and FIX, a decreased level of FXII and dysfibrinogenemia [3]. A lack of coagulation inhibitors implies rare but strong thrombophilias. Mutations in Factor Leiden and F2 gene account for up to 70% of diagnosed hereditary thrombophilias and these are considered significant thrombophilias [3]. According to the current recommendations, polymorphisms in the *methylenetetrahydrofolate reductase* (MTHFR) and plasminogen activator inhibitor-1 (PAI-1) are not considered hereditary thrombophilias, although these two polymorphisms used to be tested as part of laboratory tests for thrombophilia [3-5].

Pregnancy is physiologically characterized by hypercoagulability due to changes in homeostasis during pregnancy and postpartum. Hypercoagulability is a consequence of changes in coagulation and fibrinolytic system which are manifested by an increase in coagulation factors (factors II, VII, VIII, X, XII) and fibrinogen, i.e., increased fibrin formation and a reduced fibrinolytic activity [6]. Recent research has shown that thrombophilia can heighten the negative effects of these changes, and in addition to maternal complications it can also cause problems related to pregnancy itself: fetal loss, fetal growth retardation (IUGR), preeclampsia, and fetal death [7,8].

Recurrent pregnancy loss is not only an emotional issue, but a social and economic problem as well [9,10]. It is believed that fetal loss in early stages of pregnancy can result from various factors including anatomical abnormalities of the uterus, karyotypic abnormalities, and a defect in progesterone production. Additionally, hematological disorders such as the presence of antiphospholipid antibodies and congenital thrombophilia are considered significant contributors to fetal loss [11]. A successful pregnancy outcome depends on the development of adequate placental circulation. The placenta has dual circulation: maternal, in the intervillous space, and fetal, which consists of the chorionic vessels and chorionic villi. The success of pregnancy depends on the mechanisms that prevent coagulation in villous and fetal blood circulation [6]. If there is hypercoagulability on the part of the mother, there is a reduced flow in the intervillous space, with a high risk for the formation and deposition of fibrin deposits in the placenta

biditet. Kod trudnica sa trombofilijom disfunkcija endotelnih ćelija, aktivacija koagulacije, odlaganje fibrina i utero-placentarna tromboza dovode do smanjenja invazije trofoblasta u spiralne arterije i najzad dovode do poremećaja implantacije, ponavljajućih abortusa, IUGR, preeklampsije i fetalne smrti [12,13].

Krajem trećeg meeseca trudnoće stvara se placenta. Placentalne ćelije na svojoj površini ispoljavaju antikoagulantni sistem trombomodulin-protein C-endotelni protein C receptor. Ovaj sistem predstavlja veoma značajan inhibitorni sistem koagulacije ali i receptor za tkivni faktor [14]. Za adekvatnu placentaciju i embrionalni razvoj od velike je važnosti aktivnost antikoagulantnog sistema proteina C, jer on kontroliše trombin stvoren na površini trofoblasta ili interviloznih prostora. Stvaranje trombina je precizno kontrolisani proces ograničen samo na stvaranje ograničene količine trombina koja je dovoljna da obrazuje krvni ugrušak na mestu povrede krvnog suda. Fiziološki inhibtori trombina, od kojih je najvažniji antitrombin, inaktivišu sav višak trombina. Sklonost tromboziranju kod većine urođenih trombofilnih stanja nastaje ili zbog povećanog stvaranja trombina ili zbog nemogućnosti njegove brze i efikasne inaktivacije [15]. Nedostatak antitrombina dovodi do smanjene inaktivacije trombina, FXa FXIa i FIXa, što dovodi do povećane koncentracije aktiviranih faktora koagulacije i hiperkoagulabilnosti krvi [15]. U kontroli stvaranja trombina značajno mesto zauzima sistem proteina C. Aktivirani protein C u prisustvu kofaktora, proteina S, inaktiviše aktivne faktore V i VIII. Slobodni protein S ima i antikoagulantni efekat tako što inaktiviše i protrombinazu (kompleks FXa, FVa i fosfolipida). U slučaju nedostatka proteina C ili proteina S dolazi do povećanog stvaranja trombina, jer nedostaju mehanizmi koji to sprečavaju [15]. Stvoreni trombin negativno utiče na embrionalni razvoj jer dovodi do stvaranja fibrin degradacionih produkata, koji podstiču ćelijsku apoptozu trofoblasta.

Gubitak trudnoće jedan je od vodećih zdravstvenih problema koji se javlja kod žena u fertilnom periodu. Incidencija pobačaja kod klinički prepoznate trudnoće kreće se između 8% i 20%, dok je incidencija u razdoblju preimplantacije i rane implantacije veća i iznosi zmeđu 13% i 26% [16,17]. Tri ili više uzastopnih spontanih pobačaja s istim partnerom bez ostvarene ijedne trudnoće naziva se primarni habitualni pobačaj, dok se sekundarni habitualni pobačaj definiše kao pobačaj kod žena koje su prethodno imale uspešnu trudnoću. Verovatnoća da žena koja je imala primarni habitualni pobačaj rodi živo dete, bez lečenja, je 25-45%.

Ispitivanje prisustva trombofilnog stanja postala je sve raširenija laboratorijska praksa. Laboratorijskim ispitivanjem moguće je isključiti samo poznate uzroke

that can lead to placental infarction. If more than 5% of the placental mass is affected, perinatal morbidity is increased. In pregnant women with thrombophilia, endothelial cell dysfunction, coagulation activation, fibrin deposition, and utero-placental thrombosis lead to decreased trophoblast invasion into spiral arteries and ultimately lead to implantation disorders, recurrent abortions, IUGR, preeclampsia, and fetal death [12,13].

The placenta is formed at the end of the third month of pregnancy. Placental cells express the anticoagulant system comprising thrombomodulin, protein C, and the endothelial protein C receptor on their surface. This system is a very important inhibitory coagulation system, but also a receptor for tissue factor [14]. For adequate placentation and embryonic development, the activity of the protein C anticoagulant is of great importance as it controls the thrombin generated on the surface of the trophoblast or the intervillous space. Thrombin generation is a precisely controlled process limited to the generation of a limited amount of thrombin sufficient to form a blood clot at the site of a vascular trauma. Physiological thrombin inhibitors, with antithrombin being the most important among them, deactivate all excess thrombin. The thrombotic tendency in most congenital thrombophilic conditions arises either from heightened thrombin production or the inability to promptly and efficiently deactivate it [15]. Antithrombin deficiency results in diminished inhibition of thrombin, FXa, FXIa and FIXa, which leads to elevated levels of activated coagulation factors and blood hypercoagulability [15]. The protein C system takes an important place in the control of thrombin formation. In the presence of its co-factor protein S, activated protein C deactivates factors V and VIII. Additionally, free protein S exerts an anticoagulant effect by neutralizing prothrombinase, which consists of FXa, FVa and phospholipids. In case of protein C or protein S deficiency, thrombin generation increases due to the absence of regulatory mechanisms that would inhibit its production [15]. The generated thrombin has a negative effect on embryonic development by inducing the formation of fibrin degradation products, which, in turn, trigger cell apoptosis in the trophoblast.

Pregnancy loss is one of the leading health concerns affecting women during their fertile years. The rate of miscarriage in clinically recognized pregnancies ranges between 8% and 20%, whereas during pre-implantation and early implantation period it is higher and ranges between 13% and 26% [16,17]. Three or more consecutive spontaneous abortions with the same partner without achieving a single pregnancy are called primary habitual abortions, while secondary habitual abortions are defined as abortions in women

urođene trombofilije, ali negativan rezultat testiranja ne znači odsustvo nasledne sklonosti trombozi ranju. Kontraverzna su mišljenja o povezanosti nasledne trombofilije s pojavom spontanih pobačaja. Većina retrospektivnih studija je utvrdila postojanje skromne povezanost nasledne trombofilije i komplikacija tokom trudnoći, dok prospektivne studije opovrgavaju ovakav vid povezanosti [18-20].

Cilj rada je bio prikupljanje i analiza podataka o učestalosti urođene trombofilije kod ispitanica sa gubitkom ploda, analiza perioda trudnoće i godina života kada je došlo do gubitka ploda, i učestalosti uspešnih trudnoća nakon profilaktičke primene NMH kod ispitanica sa urođenom trombofilijom i prethodnim spontanim gubicima ploda.

METODE

Istraživanje učestalosti trombofilija kod ispitanica s gubitkom ploda je sprovedeno na Klinici za ginekologiju i akušerstvo Univerzitetskog kliničkog centra Republike Srpske. Istraživanje je dizajnirano kao retrospektivna opservacijska studija u trajanju 30 meseci, kojom su obuhvaćene ispitanice (n=69) koje su lečene na Klinici za ginekologiju i akušerstvo Univerzitetskog kliničkog centra Republike Srpske, a koje su imale jedan ili više gubitaka anatomski normalnog ploda u drugom i trećem trimestru trudnoće, ili dva i više ponavljanih gubitaka ploda u prvom trimestru trudnoće. Kod svih ispitanica su urađeni testovi za dokazivanje prisustva trombofilije, ali su urađene i druge dijagnostičke analize, radi isključivanja ostalih uzroka pobačaja: hormonski, infektivni ili autoimuni.

Analizom su obuhvaćeni podaci ispitanica dobijeni iz medicinske dokumentacije, i to sledeći: demografski, podaci o prethodnim trudnoćama i njihovom ishodu, podaci o komplikacijama u toku prethodnih trudnoća, a koji se odnose na ponovljene spontane pobačaje, intrauterino zaostajanje u rastu i razvoju, intrauterina smrt ploda, pojava abrupcije placente ili preaklampsije, i dijagnostikovana oboljenja. Takođe, istraživanjem su obuhvaćeni laboratorijski parametri: broj leukocita-Le (x10^9/l), broj trombocita-Tr (x10^9/l), hemoglobin-Hb (g/l), PT, aktivirano parcijalno tromboplastinsko vrijeme (aPTT), i fibrinogen (µg/ml). Metode rađene za dokazivanje prirodnih inhibitora su obuhvatile: funkcionalne i imunološke metode za određivanje koncentracije AT (referentne vrednosti 70-120%), funkcionalne i hromogene metode za određivanje koncentracije proteina C (referentne vrednosti 70-120%) i funkcionalne metode za određivanje proteina S (referentne vrednosti 60-120%). PCR testovi analize DNK uz dokazivanje specifične mutacije su korišćeni da bi se odredilo prisustvo mutacija: heterozigot/homozigot za faktor V

who previously had a successful pregnancy. The likelihood of a woman, without treatment, who has experienced a primary habitual abortion delivering a live child is approximately 25-45%.

The laboratory practice of testing for thrombophilic conditions has become increasingly common. With laboratory testing, it is possible to rule out only known causes of congenital thrombophilia, but a negative result does not mean the absence of a hereditary tendency to thrombosis. There are controversial opinions about the connection between hereditary thrombophilia and the occurrence of spontaneous abortions [18-20].

The objective of this study was to gather and analyze data regarding the prevalence of congenital thrombophilia among individuals experiencing fetal loss. Additionally, it aimed to examine the timing of pregnancy loss and the age range during which it occurred, as well as to assess the rate of successful pregnancies following the prophylactic administration of LMWH in individuals with congenital thrombophilia and a history of spontaneous fetal losses.

METHODS

The study of the prevalence of thrombophilia among participants experiencing fetal loss was carried out at the Clinic for Gynecology and Obstetrics of the University Clinical Centre of the Republic of Srpska. The research was designed as a retrospective observational study spanning 30 months. It included 69 participants treated at the Clinic for Gynecology and Obstetrics of the University Clinical Centre of the Republic of Srpska. These participants experienced either one or more losses of anatomically normal fetuses in the second and third trimesters of pregnancy, or two or more recurrent fetal losses in the first trimester. All participants were tested to prove the presence of thrombophilia, but other diagnostic analyses were also performed to rule out other causes of miscarriages, i.e., hormonal, infectious and autoimmune.

The analysis incorporated subject data extracted from medical records: demographics, information on prior pregnancies and their outcomes, details on complications during previous pregnancies such as recurrent spontaneous abortions, intrauterine growth restriction, intrauterine fetal demise, incidences of placental abruption or preeclampsia, as well as diagnosed medical conditions. Additionally, the research encompassed the following laboratory parameters: counts of leucocytes (Le) in x10^9/l, platelets (Tr) in x10^9/l, hemoglobin (Hb) in g/l, as well as measurements of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen levels (µg/ml). The methods employed to assess natural inhibitors incuded

Leiden, heterozigot/homozigot F2, heterozigot/homozigot MTHFR C677T, 4G/4G PAI-1, 4G/5G PAI-1 i 5G/5G PAI-1.

Svi podaci su uneti u elektronsku bazu podataka. Na osnovu dobijenih podataka i prema abecednom redu sastavljene su liste uključenih ispitanica, koje su unete u bazu podataka. Svakoj ispitanici je dodeljena određena šifra, kako bi se obezbedila anonimnost i izbeglo dupliranje podataka.

Podaci su obrađeni metodama deskriptivne statistike: aritmetička sredina, vrednost medijane, raspon varijacije (min-max), standarna devijacija i učestalost izražena apsolutnim brojevima i procentima.

REZULTATI

U ispitivanju su analizirane medicinske istorije kod ukupno 69 ispitanica starosne dobi od 20 do 46 godina. Prosečna starost ispitanica je bila 30,7 godina godina, a prethodno su imale ukupno 167 neuspešnih trudnoća, od čega su dve bile abrupcije placente u 36. i 37. nedelji trudnoće uz mrtvorođeni plod kod iste ispitanice.

Trombofilija je dokazana kod ukupno 40 ispitanica (58%). U Tabeli 1 prikazane su demografske karakteristike svih ispitanica sa spontanim pobačajem, dok je u Tabeli 2 prikazana starosna distribucija svih ispitanica sa trombofilijom i polimorfizmima MTHFRi PAI-1 i ispitanica bez dokazane trombofilije ili polimorfizma (kontrolna grupa – KG) koje su imale spontane pobačaje. U skladu s rezultatima, najveći broj neuspelih trudnoća je bio u grupi s najvećim brojem ispitanica, međutim, ako se izračuna koliko je bilo pobačaja po svakoj ispitanici u grupi, grupa starijih ispitanica sa trombofilijom (>35 godina) je u proseku imala veći broj pobačaja, 2,83 po ispitanici. Takođe i u kontrolnoj grupi ispitanica najveći broj pobačaja je bio u starosnoj grupi preko 35 godina (3,0 po ispitanici).

Rezultati su pokazali da su se kombinovane trombofilije (uključujući kombinovane polimorfizme i AFLS) javljale s najvećom učestalošću 47,5% (n=19). Manja

Tabela 1. Demografske karakteristike svih ispitanica sa gubitkom ploda

Table 1. Demographic characteristics of all participants experiencing fetal loss

Demografske karakteristike / *Demographic characteristics*	**Ukupno / *Total* (n=69)**
Godine života (godine±SD) / *Age (years±SD)*	30.7 (±5.0)
Godine života (rang) / *Age (range)*	20-46
Broj spontanih pobačaja (ukupno) / *The overall number of spontaneous abortions*	167
Broj žena s trombofilijom / *The number of women with thrombophilia*	40
Broj žena sa VTE tokom trudnoće / *The number of women with VTE during pregnancy*	1 (plućna tromboembolija / *pulmonary embolism*)

functional and immunological methodologies to ascertain AT concentration (with reference values of 70-120%), functional and chromogenic assays to determine protein C concentration (with reference values of 70-120%), and functional assays for assessing protein S concentration (with reference values of 60-120%). Mutation-specific PCR tests were used to identify mutations including heterozygous/homozygous for factor V Leiden, heterozygous/homozygous for F2, heterozygous/homozygous for MTHFR C677T, as well as variants 4G/4G, 4G/5G, and 5G/5G of PAI-1.

All data were entered into an electronic database. Lists of respondents were compiled based on the obtained data and respecting the alphabetical order and then they were entered into the database. Each respondent was assigned a specific code to ensure anonymity and avoid data duplication.

Data were processed using descriptive statistical methods including calculation of the arithmetic mean, median value, range of variation (min-max), standard deviation, and frequency expressed in both absolute numbers and percentages.

RESULTS

The study involved the analysis of medical histories from a total of 69 participants, aged 20 to 46 years. The average age of the participants was 30.7 years. Collectively, they experienced a total of 167 unsuccessful pregnancies, including two instances of placental abruption occurring in the 36th and 37th weeks of pregnancy, resulting in stillborn fetuses in the same participant.

Thrombophilia was found in a total of 40 participants (58%). Table 1 displays the demographic characteristics of all subjects with spontaneous abortion, whereas Table 2 shows the age distribution among subjects with thrombophilia and MTHFR and PAI-1 polymorphisms, along with subjects in the control group (CG) lacking confirmed thrombophilia or polymorphism but experiencing spontaneous abortions. Based on the results, the highest number of unsuccessful pregnancies was found in the largest group of respondents. However, having calculated the average number of abortions per respondent within each group, it was observed that the group of older subjects with thrombophilia (>35 years) had a higher average number of abortions, 2.83 per respondent. Additionally, within the control group of respondents, the highest number of abortions occurred among those aged over 35 years (3.0 per respondent).

The results showed that combined thrombophilias (including combined polymorphisms and APS) occurred with the highest frequency of 47.5% (n=19). A lower frequency was associated with the isolated poly-

Tabela 2. Distribucija svih ispitanica u odnosu na starosnu grupu

Table 2. Distribution of all participants categorized by age group

Dob / *Age*	Broj ispitanica / *Number of participants*		%		Broj P[1] / *Number of SA[1]*		% (od ukupno SP / *of the total SA)*	
	T/P[2]	**CG[3]**	**T/P**	**CG**	**T/P**	**CG**	**T/P**	**CG**
<30 godina / *<30 years*	18	7	45	24	40	15	24.0	8.9
30 -35godina / *30-35 years*	16	10	40	35	37	22	22.2	13.2
>35 godina / *>35 years*	6	12	15	41	17	36	9.1	21.6
Ukupno / *Total*	**40**	**29**	**100**	**100**	**94**	**73**	**56.3**	**43.7**
	69		100		167		100	

[1]SP- spontani pobačaj; [2]T/P-ispitanice sa trombofilijom i polimorfizmom MTHFR i PAI-1; [3]KG kontrolna grupa (bez trombofilije i polimorfizma MTHFR i PAI-1)

[1]SP- spontaneous abortion; [2]T/P- participants with thrombophilia and polymorphism MTHFR and PAI-1; [3]CG control group (without thrombophilia and polymorphisms of MTHFR and PAI-1)

učestalost se odnosila na izolovani polimorfizam za PAI-1 kod 27.5% (n=11), dok je mutacija s najmanjom zastupljenošću bila FV Leiden kod svega 5,0% ispitanica (n=2), izolovani homozigot MTHFR 7,5% (n=3) i MTHFR heterozigot 12.5% (n=5). Kod četiri ispitanice je utvrđen nedostatak proteina S u kombinaciji sa drugim trombofilnim stanjima. Detaljan prikaz učestalosti svih kombinovanih trombofilija je dat u Tabeli 2.

Istraživanje je pokazalo da je u grupi ispitanica sa detektovanim PAI-1, najveći broj imao detektovan 4G/4G polimorfizam (n=6), dok je 5 ispitanica imalo 4G/5G. Ukupan broj spontanih pobačaja u ovoj grupi iznosio je 25, pri čemu je 20 bilo u prvom trimestru trudnoće, dva u drugom trimestru trudnoće i 3 neuspešne trudnoće u trećem trimestru, pri čemu se jedna odnosi na mrtvorođeni plod u 32. nedelji trudnoće i dve abrupcije placente uz mrtvorođeni plod u 36. i 37. nedelji trudnoće kod ispitanice s 4G/5G polimorfizmom. Broj spontanih pobačaja u grupi sa 4G/4G PAI-1 bio je 2,33 po ispitanici, a u grupi 4G/5G PAI-1 2,4 po ispitanici.

Evidentirane su dve ispitanice sa heterozigotnom mutacijom za FV Leiden koje su imale po jednu neuspešnu trudnoću koja se završila u prvom i drugom trimestru.

Izolovana homozigotna mutacija za MTHFR je evidentirana kod 3 ispitanice. Dve ispitanice su imale povišene vrednosti homocisteina, a kod jedne su vrednosti bile na gornjoj granici referentnog. U ovoj grupi je utvrđeno ukupno 7 neuspešnih trudnoća, od kojih se 6 završilo u prvom trimestru trudnoće, a jedna u drugom.

U grupi ispitanica sa naslednom trombofilijom primenjen je NMH kod 22 ispitanice. Kod svih ispitanica lek je primenjen kao profilaktička antikoagulantna terapija zbog prethodnih neuspešnih trudnoća. Uspešan ishod trudnoće nakon primene NMH evidentiran je kod 19 ispitanica. Kod 3 ispitanice je bez obzira na primenjeni lek došlo do gubitka ploda, i to kod dve u drugom trimestru trudnoće i kod jedne ispitanice u trećem trimestru trudnoće.

morphism for PAI-1, found in 27.5% of subjects (n=11). The mutations with the lowest prevalence were FV Leiden, detected in only 5.0% of subjects (n=2), isolated homozygous MTHFR, observed in 7,5% of subjects (n=3), and MTHFR heterozygous, present in 12.5% of subjects (n=5). Protein S deficiency in combination with other thrombophilic conditions was found in four subjects. A detailed overview of the frequency of all combined thrombophilias is shown in Table 2.

The study revealed that within the group of test subjects with detected PAI-1, the majority exhibited the 4G/4G polymorphism (n=6), whereas five test subjects showed the 4G/5G variant. In this group, there were a total of 25 spontaneous abortions, with 20 occurring in the first trimester, two in the second trimester, and three in the third trimester. Among the latter, one involved a stillborn fetus at 32 weeks of gestation, while two were placental abruptions resulting in stillborn fetuses – one at 36 weeks and the other at 37 weeks – both observed in a subject with the 4G/5G polymorphism. The number of spontaneous abortions in the 4G/4G PAI-1 group was 2.33 per test subject, and in the 4G/5G PAI-1 there were 2.4 spontaneous abortions per test subject.

Two test subjects with a heterozygous mutation for FV Leiden were identified, each experiencing one unsuccessful pregnancy that terminated in the first and second trimesters, respectively.

Isolated homozygous mutation for MTHFR was found in three subjects. Two subjects exhibited elevated homocysteine levels, with one subject's values reaching the upper limit of the reference range. A total of 7 unsuccessful pregnancies were recorded in this group, 6 of which ended in the first trimester, and one in the second trimester.

In the group of subjects with hereditary thrombophilia, LMWH was applied to 22 subjects. In all subjects, the drug was administered as prophylactic anticoagulant therapy due to previous unsuccessful pregnan-

Tabela 3. Kombinovane trombofilije i polimorfizmi MTHFR I PAI-1 kod ispitanica sa spontanim pobačajima

Table 3. Combined thrombophilias and polymorphisms of MTHFR and PAI-1 in participants with spontaneous abortions

Kombinovane trombofilije i polimorfizmi / *Combined thrombophilias and polymorphisms*	**Broj ispitanica / *The number of participant***	**Spontani pobačaji / *Spontaneous abortions***	
		I trimester / *I trimester*	**II trimester / *II trimester***
MTHFR homozigot+4G/4G PAI-1 / *MTHFR homozygous +4G/4G PAI-1*	4	11	
MTHFR heterozigot+4G/5G PAI-1 / *MTHFR heterozygous +4G/5G PAI-1*	1	3	
AFLS+4G/4G PAI-1 / *APS+4G/4G PAI-1*	2	3	2
AFLS+4G/5G PAI-1 / *APS+4G/5G PAI-1*	3	7	3
AFLS+4G/5G PAI-1+MTHFR heterozigot / *APS+4G/5G PAI-1+MTHFR heterozygous*	1	2	
AFLS+MTHFR homozigot / *APS+MTHFR homozygous*	2	5	1
AFLS+4G/4G PAI-1+nedostatak FXII / *APS+4G/4G PAI-1+a lack of FXII*	1		1
PS+MTHFR homozigot+4G/4G PAI-1 / *PS+MTHFR homozygous +4G/4G PAI-1*	1	1	1
PS+nedostatak FXII / *PS+ a lack of FXII*	1		1
PS+4G/4G PAI-1 / *PS+4G/4G PAI-1*	1	2	
PS+4G/5G PAI-1+ FV Leiden heterozigot / *PS+4G/5G PAI-1+ FV Leiden heterozygous*	1		
F2+FV Leiden heterozigot+MTHFR heterozigot+4G/5GPAI-1 / *F2+FV Leiden heterozygous + MTHFR heterozygous +4G/5GPAI-1*	1	3	
Ukupno / *Total*	19 (27.5%)	37	9
		47	

U grupi ispitanica s kombinovanom trombofilijom evidentirano je ukupno 47 neuspešnih trudnoća, od kojih je jedna bila u trećem trimestru trudnoće, devet u drugom trimestru, dok su se preostale trudnoće završile u prvom trimestru trudnoće. Kod 9 ispitanica je primenjen NMH u profilaktičkoj dozi. Terapija je uključena u periodu od 5. do 19. nedelje trudnoće. Kod jedne ispitanice kod koje je terapija uvedena u 19. nedelji trudnoće, neposredno nakon testiranja na urođene trombofilije, došlo je do gubitka ploda u 22. nedelji trudnoće. Kod preostalih osam ispitanica trudnoća se završila bez komplikacija, terminskim porođajima. U Tabeli 3 prikazani su ishodi trudnoće kod 9 ispitanica sa

cies. A successful pregnancy outcome upon the application of LMWH was observed in 19 subjects. In three subjects, fetal loss occurred irrespective of the administered drug, with two cases observed in the second trimester of pregnancy and one in the third trimester.

In the group of subjects with combined thrombophilia, a total of 47 unsuccessful pregnancies were recorded, of which one occurred in the third trimester of pregnancy, 9 in the second trimester, while the remaining unsuccessful pregnancies occurred in the first trimester of pregnancy. In 9 subjects, LMWH was administered in a prophylactic dose. The therapy was administered during the period spanning from the

Tabela 4. Primenjena NMH i ishodi trudnoće kod kombinovane trombofilije i polimorfizama MTHFR i PAI-1

Table 4. Applied LMWH and pregnancy outcomes in combined thrombophilia and polymorphism of MTHFR and PAI-1

Ispitanice sa kombinovanom trombofilijom i polimorfizmom / *Participants with combined thrombophilia and polymorphism*			**GN početka primjene LMWH / *Week of gestation (WG) at the beginning of LMWH administration***	**Ishodi trudnoće / *Pregnancy outcomes***	
Tip / *Tip*	**Broj / *Number***	***SP / SA***		**Porođaj / *Delivery***	**GN pobačaj / *WG abortion***
AFLS+4G/4G PAI-1 / *APS + 4G/4G PAI-1*	1	3	19		22
AFLS+4G/5G PAI-1 / *APS + 4G/5G PAI-1*	1	4	5	37 WG	
AFLS +MTHFR homozigot / *AFLS + MTHFR homozygous*	1	3	14	37 WG	
PS+MTHFR homozigot + 4G/4G PAI-1 / *PS+MTHFR homozygous + 4G/4G PAI-1*	1	2	7	39 WG	
PS+4G/4G PAI-1 / *PS+4G/4G PAI-1*	1	2	16	38 WG	

kombinovanom trombofilijom koje su lečene profilaktičkom dozom NMH.

U grupi ispitanica kod kojih je utvrđen izolovani polimorfizam za PAI-1 utvrđeno je ukupno 25 neuspešnih trudnoća (2,27 pobačaja po ispitanici). Kod svih ispitanica je pri narednoj trudnoći uveden NMH u profilaktičkoj dozi uz uspešne ishode trudnoća kod 9 ispitanica, dok je kod 2 ispitanice bez obzira na primenjeni lek došlo do spontanog gubitka ploda (Tabela 4). Broj pobačaja po ispitanici pod terapijom NMH iznosio je 0,18 što je značajno manje u odnosu na broj pobačaja po ispitanici bez primenjene terapije NMH (2,27).

Istraživanje je pokazalo da je u grupi ispitanica s homozigotnom MTHFR mutacijom (n=3) prethodno bilo 7 neuspešnih trudnoća. Kod dve ispitanice je započeta terapija NMH do 12. nedelje trudnoće. U oba slučaja je došlo do povoljnog ishoda trudnoće. U periodu praćenja tokom ovog istraživanja, nije bilo ostvarenih trudnoća kod ispitanica sa utvrđenom heterozigotnom mutacijom za FV Leiden.

U grupi ispitanica s trombofilijom urađena je komparacija ishoda trudnoće pre utvrđene nasledne trombofilije sa ishodima trudnoće nakon što je primenjena terapija u periodu praćenja. Kod 40 ispitanica s trombofilijom prethodno je bilo ukupno 94 neuspešne trudnoće i jedna plućna tromboembolija (PTE) tokom trudnoće. U toku praćenja, trudnoća je ostvarena kod 22 ispitanice. U ovoj grupi (n=22) evidentirano je ukupno 59 nepovoljnih ishoda trudnoće i jedna plućna tromboembolija tokom trudnoće. Kod svih ispitanica primenjivan je NMH. Kod 20 ispitanica trudnoća se održala i završena je porođajem, a nepovoljan ishod trudnoće je evidentiran kod 3 ispitanice (Tabela 5).

U grupi ispitanica koje nisu imale dokazanu trombofiliju u posmatranom periodu bilo je 8 uspešnih porođaja. Utvrđena je statistički značajna razlika ($p < 0,05$) između broja porođaja kod ispitanica sa kombinovanom trombofilijom i kombinovanim polimorfizmima koje su lečene NMH i grupe ispitanica koje nisu

5th to the 19th week of pregnancy. In one subject, who started therapy in the 19th week of pregnancy immediately after testing for congenital thrombophilia, fetal loss occurred in the 22nd week of pregnancy. In the remaining eight subjects, the pregnancies concluded without any complications, resulting in full-term births. Table 3 shows the pregnancy outcomes in 9 subjects with combined thrombophilia who were treated with a prophylactic dose of LMWH.

A total of 25 unsuccessful pregnancies (2.27 abortions per test subject) were observed in the group of subjects with isolated polymorphism for PAI-1. In all cases, LMWH was administered prophylactically during subsequent pregnancies. The outcomes were successful in nine subjects, while two subjects experienced spontaneous fetal loss regardless of the medication administered, as shown in Table 4. The average number of abortions per subject under LMWH therapy was 0.18, markedly lower than the average number of abortions per subject without LMWH therapy, which was 2.27.

The research showed that among the group of subjects with a homozygous MTHFR mutation (n=3), there were a total of 7 unsuccessful pregnancies prior to the study. In two subjects LMWH therapy was initiated up to the 12th week of pregnancy. In both cases pregnancies were successful. Throughout the follow-up period of this study, no pregnancies occurred in subjects with a confirmed heterozygous mutation for FV Leiden.

The study compared pregnancy outcomes in the group of subjects with thrombophilia before the diagnosis of hereditary thrombophilia was established with pregnancy outcomes after the therapy was administered during the follow-up period. Among the 40 subjects with thrombophilia, there were a total of 94 previous unsuccessful pregnancies and one instance of pulmonary thromboembolism (PTE) during pregnancy. During the follow-up period, pregnancy occurred in 22 test subjects. A total of 59 adverse pregnancy outcomes and one case of pulmonary thromboembolism

Tabela 5. Primenjena NMH i ishodi trudnoće kod kombinovanih polimorfizama MTHFR i PAI-1

Table 5. Administered LMWH and pregnancy outcomes in combined polymorphisms MTHFR and PAI-1

Ispitanice sa kombinovanim polimorfizmom / *Participants with combined polymorphism*			**GN početka primjene LMWH / *WG at the beginning of LMWH administration***	**Ishodi trudnoće / *Pregnancy outcomes***	
Tip / *Tip*	**Broj / *Number***	**SP / *SA***		**Porođaj / *Delivery***	**GN pobačaj / *WG abortion***
MTHFR homozigot+ 4G/4G PAI-1 / *MTHFR homozygous + 4G/4G PAI-1*	1	2	6	38 WG	
MTHFR homozigot+ 4G/4G PAI-1 / *MTHFR homozygous + 4G/4G PAI-1*	1	2	8	36 WG	
MTHFR homozigot+ 4G/4G PAI-1 / *MTHFR homozygous + 4G/4G PAI-1*	1	2	8	38 WG	
MTHFR homozigot+ 4G/5G PAI-1 / *MTHFR homozygous + 4G/5G PAI-1*	1	3	12	39 WG	

AFLS-antifosfolipidni sindrom; NMH-heparin male molekulske težine; GN-nedelja gestacije; SP-spontani pobačaj

APS – antiphospholipid syndrome; LMWH – low molecular weight heparin; WG – week of gestation; SA – spontaneous abortion

Tabela 6. Komparacija ishoda trudnoće pre i nakon primene NMH kod ispitanica sa trombofilijom i polimorfizmima MTHFR i PAI-1

Table 6. Comparison of pregnancy outcomes before and after LMWH administration in participants with thrombophilia and polymorphism of MTHFR and PAI-1

Ispitanice sa trombofilijom ili polimorfizmom MTHFR /PAI-1 / *Participants with thrombophilia and polymorphism of MTHFR/PAI-1*		**Prethodne trudnoće / *Prior pregnancies***		**Ishodi trudnoće / *Pregnancy outcomes***	
Tip / *Type*	**Broj / *Number***	**Pobačaj / *Abortion***	**VTE[1] / *VTE[1]***	**Porođaj / *Delivery***	**Pobačaj / *Abortion***
Kombinovane trombofilije / *Combined thrombophilias*	5	14	0	5	0
MTHFR homozigot+4G/5GPAI-1 / *MTHFR homozygous +4G/5GPAI-1*	1	3	0	1	0
MTHFR homozigot+4G/4GPAI-1 / *MTHFR homozygous +4G/4GPAI-1*	3	11	0	3	1
MTHFR homozigot / *MTHFR homozygous*	2	6	1	2	0
4G/4G PAI-1 / *4G/4G PAI-1*	6	13	0	5	1
4G/5G PAI-1 / *4G/5G PAI-1*	5	12	0	4	1
Ukupno /*Total*	**22**	**59**	**1**	**20**	**3**

[1]VTE-venski tromboembolizam

[1]VTE-venous thromboembolism

imale trombofiliju (OR = 3,179; 95% CI = 1,071-9,405; p = 0,037), ali nije utvrđena statistički značajna razlika između broja porođaja kod ispitanica sa kombinovanom trombofilijom (ukoliko se isključe polimorfizmi MTHFR i PAI-1) koje su lečene NMH i grupe ispitanica koje nisu imale trombofiliju (OR=2,607; 95% CI= 0,689-9,853; p = 0,158). Nije utvrđena statistički značajna razlika (p > 0,05) između broja uspešnih porođaja u grupi svih ispitanica sa trombofilijom u odnosu na grupu ispitanica bez dokazane trombofilije (OR=1,941; 95% CI=0,809-4,658; p = 0,137), ali je postojala statistički značajna razlika između broja porođaja u grupi pacijentkinja koje su koristile NMH i kontrolne grupe (OR=3,093; 95% CI=1,272-7,524; p = 0,013).

DISKUSIJA

Reproduktivni neuspeh nosi značajne lične i društvene posledice i ima veliki uticaj na javno zdravlje. Pored emocionalnog problema, ponavljani gubitak trudnoće predstavlja i socijalno-ekonomski problem društva [20].

U ovom istraživanju je kod 58% pacijentkinja utvrđeno prisustvo nasledne trombofilije. Kupferminic i saradnici su u svom istraživanju, u kome su pratili 100 ispitanica sa neobjašnjenim ponavljanim gubitkom trudnoće i 43 ispitanice iz kontrolne grupe, utvrdili visoko prisustvo trombofilije kod ukupno 65% ispitanica iz prve grupe naspram 18% ispitanica s trombofilijom iz kontrolne grupe [21]. U HABENOX studiji Visera i saradnika trombofilija je kao uzrok ponovljenih spontanih pobačaja identifikovana kod 50% ispitanica, koje su imale tri ili više spontanih pobačaja u prvom trimestru trudnoće ili dva ili više spontanih pobačaja u drugom i trećem trimestru trudnoće ili jedan sponatni pobačaj u trećem i jedan u prvom trimestru trudnoće [22]. Slične rezultate imali su Brener i saradnici u istraživanju u kome je analiziran uzrok spontanih pobačaja kod 76

during pregnancy were recorded in this group (n=22). LMWH was administered to all subjects. In 20 subjects, the pregnancies were carried to term and concluded with childbirth, while unfavorable pregnancy outcomes were recorded in 3 subjects, as shown in Table 5.

There were 8 successful deliveries in the group of subjects who did not have confirmed thrombophilia in the period of observation. A statistically significant difference (p < 0.05) was observed in the number of deliveries between subjects with combined thrombophilia and combined polymorphisms who received LMWH treatment and the group of subjects without thrombophilia (OR=3.179; 95% CI=1.071-9.405; p = 0.037). However, no statistically significant difference was found in the number of deliveries between subjects with combined thrombophilia (excluding MTHFR and PAI-1 polymorphisms) who received LMWH treatment and the group without thrombophilia (OR=2.607; 95% CI=0.689-9.853; p = 0.158). No statistically significant difference (p > 0.05) was observed in the number of successful deliveries between the group of all subjects with thrombophilia and the group of subjects without confirmed thrombophilia (OR=1.941; 95% CI=0.809-4.658; p = 0.137). However, a statistically significant difference was found in the number of deliveries between the group of patients who used LMWH and the control group (OR=3.093; 95% CI=1.272-7.524; p = 0.013).

DISCUSSION

Reproductive failure has significant personal and social consequences and a major impact on public health. Apart from being an emotional problem, recurrent pregnancy losses are also a social and economic problem [20].

In this study, the presence of hereditary thrombophilia was identified in 58% of patients. In their study,

ispitanica, koje su imale 3 ili više spontanih pobačaja u prvom trimestru trudnoće, dva ili više sponatnih pobačaja u drugom trimestru trudnoće i jedan u trećem trimestru. Ovim istraživanjem je evidentirano da je trombofilija uzrok spontanih pobačaja kod 49% ispitanica [23].

Iznenađujući je rezultat da je u ovom istraživanju, kombinovana trombofilija bila najčešći uzrok spontanih pobačaja kod 47,5% ispitanica. Najveći broj spontanih pobačaja bio je u prvom trimestru trudnoće, i predstavlja jednu trećinu svih spontanih pobačaja u grupi s trombofilijom. Kombinovane trombofilije, bilo da se radi o kombinaciji stečene i urođene trombofilije ili dve i više urođenih trombofilija, identifikovane su kao uzroci i ranih i kasnih spontanih pobačaja kod žena. U istraživanju Ivanova i saradnika u koje je bilo uključeno 52 ispitanice s gubitkom ploda između 10. i 20. nedelje trudnoće, utvrđena je učestalost kombinovanih trombofilja kod 7,7% ispitanica, pri čemu se najveća učestalost odnosila na kombinaciju FV Leiden mutacije i polimorfizma 4G/5G PAI-1 [24]. EPCOT studija (*European Prospective Cohort on Thrombophilia*) u koju je bilo uključeno 843 ispitanice s trombofilijom od kojih je 571 ispitanica imala 1524 trudnoće, u poređenju sa kontrolnom grupom u kojoj je bilo 541 ispitanica, od kojih je 395 imalo 1019 trudnoće. Stopa gubitaka trudnoće je bila veća u grupi koja je imala više od jedne utvrđene trombofilije (OR 14,3) [25].

Kod nešto više od jedne četvrtine ispitanica (27,5%) utvrđen je polimorfizam 4G/4 PAI-1 i 4G/5G PAI-1. Broj spontanih pobačaja koji je evidentiran u ovoj grupi iznosio je 25 i uglavnom se odnosio na spontane pobačaje u prvom trimestru trudnoće (n=20, 80%). U istraživanju Dozenbaka i saradnika u kome je analizirana učestalost pojedinih trombofilija u populaciji žena (n=49) koje su imale dva ili više spontanih pobačaja u prvom trimestru trudnoće, utvrđena je relativno visoka učestalost polimorfizma 4G/5G PAI-1 kod 57% ispitanica, dok je učestalost 4G/4G PAI-1 bila evidentirana kod 25% ispitanica. Poređenjem grupe ispitanica koje su imale polimorfizam 4G/4G naspram 5G/5G PAI-1 uočeno je da je relativni rizik za rani gubitak trudnoće bio značajno veći (OR 2,5), u odnosu na grupe 4G/5G naspram 5G/5G (OR 1,9) i 4G/4G u odnosu na 4G/5G PAI-1 (OR 1,3) [26]. Subrt i saradnici su u svom istraživanju utvrdili visoku, statistički značajnu povezanost između ponavljanih gubitaka trudnoće i 4G/4G PAI-1, dok za polimorfizam 4G/5G PAI-1 nisu utvrdili statistički značajnu povezanost s ponovljenim gubicima trudnoće [27].

Heterozigotna mutacija za faktor V Leiden je utvrđena kod 10% pacijentkinja. Koher i saradnici su u svom istraživanju utvrdili da postoji statistički značajna povezanost između prisustva FV Leiden mutacije

Kupferminic et al. followed 100 subjects experiencing unexplained recurrent pregnancy loss alongside 43 subjects from the control group. They discovered a significantly elevated prevalence of thrombophilia, with 65% of individuals in the first group exhibiting this condition compared to only 18% in the control group [21]. In the HABENOX study conducted by Visser et al., thrombophilia was the cause of repeated spontaneous abortions in 50% of the test subjects. These individuals experienced either three or more spontaneous abortions during the first trimester of pregnancy, or two or more during the second and third trimesters, or one in each of the first and third trimesters [22]. Brenner et al. obtained similar results in their study, analyzing the origins of spontaneous abortions in 76 subjects. These individuals had experienced three or more spontaneous abortions during the first trimester of pregnancy, two or more during the second trimester, and at least one during the third trimester. This research documented that thrombophilia accounted for 49% of spontaneous abortions among the respondents [23].

Surprisingly, in this study thrombophilia was the most common cause of spontaneous abortions in 47.5% of the respondents. The majority of spontaneous abortions occurred during the first trimester of pregnancy, constituting one-third of all such cases within the group affected by thrombophilia. Combined thrombophilias, whether arising from a combination of acquired and congenital factors or from multiple congenital conditions, have been recognized as causes of both early and late spontaneous abortions in women. In the study conducted by Ivanov et al., including 52 test subjects experiencing fetal loss between the 10th and 20th week of pregnancy, the occurrence of combined thrombophilia was identified in 7.7% of the participants. The most prevalent combination involved the FV Leiden mutation along with the 4G polymorphism/5G PAI-1 [24]. The EPCOT study (European Prospective Cohort on Thrombophilia) included 843 subjects with thrombophilia, among whom 571 individuals experienced a total of 1524 pregnancies, compared to a control group comprising 541 subjects, of whom 395 had a collective total of 1019 pregnancies. The incidence of pregnancy loss was higher in the group exhibiting multiple established thrombophilias (OR 14.3) [25].

In slightly more than a quarter of the respondents (27.5%), the 4G/4G PAI-1 and 4G/5G PAI-1polymorphisms were found. Twenty-five spontaneous abortions were recorded in this group, and those were mostly spontaneous abortions in the first trimester of pregnancy (n=20, 80%). In a study conducted by Dossenbach et al., analyzing the prevalence of specif-

i mrtvorođene dece, s OR 10,9 [28]. U NOHA studiji (*Nimes Obstetricians and Hematologists*), *case-control* dizajn studija, u koju je bilo uključeno preko 32.000 ispitanice, kod 18% ispitanica koje su imale neuspešnu trudnoću postojala je jasna povezanost s prisustvom heterozigotne mutacije za FV Leiden (OR 3,46) i mutacijom za F2 (OR 2,6) [29]. Neuspešne trudnoće su se uglavnom odnosile na gubitak ploda nakon 10. nedelje trudnoće, a nisu se mogle povezati sa ranim gubitkom trudnoće.

Brojne studije su ispitivale efikasnost primene antikoagulantne terapije u trudnoći kod ispitanica sa trombofilijom i ponovljenim gubicima ploda. Jedna od studija je uključivala 160 ispitanica sa gubitkom trudnoće nakon 10. nedelje trudnoće i dokazanim hiperkoagulabilnim stanjem: FV Leiden mutacija, F2 mutacija ili deficit proteina S. Pacijentkinje koje su primale enoksiparin imale su 86% uspešnih trudnoća u odnosu na 28% uspeešnih trudnoća u grupi kod kojih je primenjen aspirin [30]. Primena tromboprofilakse za vreme trudnoće je rezultirala nižom stopom gubitka trudnoće (0% u odnosu na 45%).

Brener i saradnici u svom istraživanju su utvrdili da primenom NMH kod ispitanica s prethodnim spontanim pobačajima i utvrđenom trombofilijom raste broj pozitivnih ishoda trudnoće, uz povećanje broja živorođene dece. Takođe, isti autor je dokazao da doza enoksiparina od 40 mg jednom dnevno je dovoljno efikasna u profilaksi spontanih pobačaja [31]. U svom istraživanju Grir daje podatke da je 85% trudnica kod kojih je primenjen NMH imalo povoljne ishode trudnoće, a Bauerzak navodi brojku od 94% [32,33]. Za razliku od ovih autora, zaključci studije ALIFE 2 su da primena NMH ne dovodi do veće stope živorođene dece kod ispitanica sa dva ili više spontana pobačaja i potvrđenom naslednom trombofilijom [34].

Jedan od nedostataka ovog istraživanja je svakako mali uzorak ispitanica, koji ukazuje na potrebu za sprovođenjem većeg istraživanja, na većem uzorku ispitanica uz duže vreme praćenja. Takođe, jedan od nedostataka je neadekvatna dijagnostička obrada pojedinih ispitanica sa ponovljenim gubicima ploda, uz postojanje određenog broja ispitanica kod kojih nisu primenjene sve dostupne dijagnostičke analize nakon ponavljanih gubitaka ploda (imunološke, patohistološka analiza nakon pobačaja, infektivni uzroci).

Ovo istraživanje daje skroman doprinos ranijim retrospektivnim istraživanjima u kojima postoji uticaj nasledne trombofilije na ishod trudnoća, jer je utvrđena učestalost uspešnih trudnoća bez komplikacija od 82% kod ispitanica sa naslednom trombofilijom, bez prethodnih porođaja, kod kojih je u trudnoći primenjen NMH.

ic thrombophilias among a cohort of women (n=49) experiencing two or more spontaneous abortions in the first trimester of pregnancy, a relatively high prevalence of the 4G/5G PAI-1 polymorphism was observed in 57% of the subjects. Meanwhile, the 4G/4G PAI-1 variant was detected in 25% of the respondents. When comparing subjects with the 4G/4G versus 5G/5G PAI-1 polymorphism, a significantly elevated relative risk for early pregnancy loss was noted (OR 2.5). In contrast, the groups 4G/5G versus 5G/5G (OR 1.9) and 4G/4G versus 4G/5G PAI-1 (OR 1.3) exhibited comparatively lower relative risks [26]. In their research, Subrt et al. found a substantial and statistically significant correlation between repeated pregnancy losses and the 4G/4G PAI-1 polymorphism. However, they did not establish a statistically significant association between recurrent pregnancy losses and the 4G/5G PAI-1 polymorphism [27].

Heterozygous mutation for Factor V Leiden was identified in 10% of patients. In their study, Koher et al. found a statistically significant association between the presence of the FV Leiden mutation and stillbirth, with an OR of 10.9 [28]. In the NOHA study (Nimes Obstetricians and Hematologists), employing a case-control design and including over 32,000 participants, there was a significant finding: 18% of individuals experiencing unsuccessful pregnancies showed a significant correlation with heterozygous mutations for FV Leiden (OR 3.46) and F2 mutations (OR 2.6) [29]. Unsuccessful pregnancies were mostly related to fetal loss after the 10th week of pregnancy and could not be associated with early pregnancy loss.

Numerous studies have investigated the effectiveness of anticoagulant therapy during pregnancy in subjects with thrombophilia and recurrent fetal losses. One of the studies included 160 subjects who experienced pregnancy loss beyond the 10th week, each exhibiting a confirmed hypercoagulable state marked by the presence of either the FV Leiden mutation, F2 mutation, or protein S deficiency. Patients administered enoxaparin experienced an 86% success rate in pregnancies, compared to the 28% success rate observed in the aspirin group [30]. The use of thromboprophylaxis during pregnancy resulted in a lower pregnancy loss rate (0% versus 45%).

In their research, Brenner et al. discovered that employing LMWH in individuals with a history of spontaneous abortions and confirmed thrombophilia not only increased the incidence of favorable pregnancy outcomes but also led to a rise in live births. Additionally, the same author proved the enoxaparin dose of 40 mg once a day was sufficiently effective in the prophylaxis of spontaneous abortions [31]. In his research,

ZAKLJUČAK

Na osnovu sprovedenog istraživanja može se zaključiti da kombinovane trombofilije nose značajno veći rizik za gubitak trudnoće kod ispitanica svih starosnih grupa. Starost preko 35 godina nosi rizik za veću učestalosti spontanih pobačaja nezavisno od tipa nasledne trombofilije. Primena NMH značajno poboljšava ishode trudnoća kod ispitanica sa urođenom trombofilijom i prethodnim gubicima trudnoće.

Sukob interesa: Nije prijavljen.

LITERATURA / *REFERENCES*

1. British Committee for Standards in Haematology. Guidelines on investigation and management of thrombophilia. J Clin Pathol. 1999;43:703-10. doi: 10.1136%2Fjcp.43.9.703.
2. Campello E., Spiezia L., Adamo A., Simioni P. Thrombophilia, risk factors and prevention. Expert Rev. Hematol. 2019;12:147-58. doi: 10.1080/17474086.2019.1583555.
3. Samfireag M, Potre C, Potre O, Tudor R, Hoinoiu T, Anghel A. Approach to Thrombophilia in Pregnancy-A Narrative Review. Medicina (Kaunas). 2022;58(5):692. doi: 10.3390%2Fmedicina58050692.
4. Deloughery TG, Beverley J, Hunt BJ, Barnes GD, Connors JM. A call to action: MTHFR polymorphisms should not be a part of inherited thrombophilia testing. Res Pract Thromb Haemost. 2022 (4):e12739. doi: 10.1002/rth2.12739.
5. Middeldorp S, Nieuwlaat R, Baumann Kreuziger L, Coppens M, Houghton D, James AH et al. American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing. Blood Adv. 2023;7(22):7101-38. doi: 10.1182/bloodadvances.2023010177.
6. Hellgren M. Hemostasis during normal pregnancy and puerperium. Semin. Thromb. Hemost. 2003;29:125-30. doi: 10.1055/s-2003-38897.
7. Simcox LE, Ormesher L, Tower C, Greer IA. Thrombophilia and Pregnancy Complications. Int. J. Mol. Sci. 2015;16:28418-28. doi: 10.3390%2Fijms161226104.
8. Ahangari N, Doosti M, Mousavifar N, Attaran M, Shahrokhzadeh S, Memarpour S, et al. Hereditary thrombophilia genetic variants in recurrent pregnancy loss. Arch. Gynecol. Obstet. 2019;300:777-82. doi: 10.1007/s00404-019-05224-7.
9. Sattar N, Greer IA, Rumley A, Stewart G, Shepherd J, Packard CJ, et al. A longitudinal study of the relationships between haemostatic, lipid and oestradiol changes during normal human pregnancy. Thromb Haemost 1999;81:71-5.
10. Bremme K. Haemostasis in normal pregnancy. In Brenner B, Mardar V, Conard J, editors. Woomens issues in thrombosis and haemostasis. Martin Dunitz Ltd, a member of the Taylor and Francis Group; 2002: 151-65.
11. Elezović I. Osnovni principi laboratorijskog pristupa u dijagnostici hemoragijskih sindroma i tromboze. U: Marisavljević (urednik) Klinička hematologija. Zavod za udžbenike, Beograd 2012: 729-39.
12. Bhave AA. Coagulopathies in Pregnancy: What an Obstetrician Ought to Know! J Obstet Gynaecol India. 2019;69:479-82. doi: 10.1007%2Fs13224-019-01290-8
13. Vodnik T, Ignjatovic S, Majkic N, Singh. Parametri hemostaze kao pokazatelji hiperkoagulabilnosti u trudnoći; Jugoslovenska medicinska biohemija 2003; 22.

Greer presents data indicating that 85% of pregnant women who used LMWH experienced positive pregnancy outcomes, whereas Bauersach cites an even higher figure of 94% [32,33]. Unlike these authors, the ALIFE 2 study concludeed that the administration of LMWH did not result in increased rates of live births among individuals with a history of two or more spontaneous abortions and confirmed hereditary thrombophilia [34].

The small sample of respondents is one of the shortcomings of this research, which indicates the need for conducting a larger study including a larger sample of respondents with an extended follow-up duration. Another shortcoming is the insufficient diagnostic treatment received by certain test subjects experiencing recurrent fetal losses. This is compounded by the fact that some participants did not undergo the full range of available diagnostic analyses following repeated fetal losses, including immunological assessments, pathohistological analyses post-abortion, and investigations into potential infectious causes.

This study is a modest contribution to previous retrospective research exploring the impact of hereditary thrombophilia on pregnancy outcomes. It revealed an 82% frequency of successful pregnancies without complications among subjects with hereditary thrombophilia, without prior births, who received LMWH during pregnancy.

CONCLUSION

Based on the conducted research, it can be concluded that combined thrombophilia poses a significantly heightened risk of pregnancy loss across all age groups. Age over 35 years is a risk for a higher frequency of spontaneous abortions, irrespective of the specific type of hereditary thrombophilia. The application of LMWH significantly improves pregnancy outcomes in subjects with congenital thrombophilia and previous pregnancy losses.

Conflict of interest: None declared.

14. Castillo MM, Yang Q, Sigala AS, McKinney DT, Zhan M, Chen KL, et al. The endothelial protein C receptor plays an essential role in the maintenance of pregnancy. Sci Adv. 2020;6(45):eabb6196. doi: 10.1126/sciadv.abb6196.
15. Miljić P. Urođena trombofilna stanja. U: Marisavljević (urednik) Klinička hematologija. Zavod za udžbenike, Beograd 2012:795-803.
16. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril 2003;79(3):577-84. doi: 10.1016/s0015-0282(02)04694-0.
17. Lohstroh PN, Overstreet JW, Stewart DR, Nakajima ST, Cragun JR, Boyers SP, et al. Secretion and excretion of human chorionic gonadotropin during early pregnancy. Fertil Steril 2005;83(4):1000-11. doi: 10.1016/j.fertnstert.2004.10.038.
18. Dizon-Townson D, Miller C, Sibai B, Spong CY, Thom E, Wendel G, et al. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. Obstet Gynecol 2005;106(3):517-24. doi: 10.1097/01.aog.0000173986.32528.ca.
19. Said JM, Higgins JR, Moses EK, Walker SP, Borg AJ, Monagle PT, et al. Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. Obstet Gynecol 2010;115(1):5-13. doi: 10.1097/aog.0b013e3181c68907.
20. Kagami M, Maruyama T, Koizumi T, Miyazaki K, Nishikawa-Uchida S, Oda H, et al. Psychological adjustment and psychosocial stress among Japanese couples with a history or recurrent pregnancy loss. Human reprod 2012;27:787-94. doi: 10.1093/humrep/der441.
21. Kupferminic MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Icreased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999;340:9-13. doi: 10.1056/nejm199901073400102.
22. Visser J, Ulander VM, Helmerhorst FM, Lampinen K, Morin-Papunen L, Bloemenkamp KWM, et al. Thromboprophylaxis for recurrent miscarriage in women with or without thrombophilia. HABENOX: a randomized multicenter trial. Thromb Hemost 2011;105:295-301. doi: 10.1160/th10-05-0334.
23. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Hemost 1999;82:6-9.
24. Ivanov P, Komsa-Penkova R, Konova E, Gecheva S, Ivanov I, Kovacheva K, et al. Combined thrombophilic factors among women with late recurrent spontaneous abortions. Akush Ginekol 2011;50:8-12.
25. Preston FE, Rosendaal FR, Walker ID, Briet E, Berntorp E, Conard J, et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996;348(9032):913-6. doi: 10.1016/s0140-6736(96)04125-6.
26. Dossenbach M, Van Trotsenburg M, Dossenbach-Glaninger A. Plasminogen activator inhibitor I 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clinical Chemistry 2003;49:1081-6. doi: 10.1373/49.7.1081.
27. Subrt I, Ulcova-Gallova Z, Bibkova K, Micanova Z, Hejnalova M, Cerna M, et al. Recurrent pregnancy loss and frequency of eight antiphospholipid antibodies and genetic thrombophilic factors in Czech women. American J of Reprod Immunolo. 2008;59:193-200. doi: 10.1111/j.1600-0897.2007.00554.x.
28. Kocher O, Cirovic C, Malynn E, Rowland CM, Bare LA, Young BA, et al. Obstetric complications in patients with hereditary thrombophilia identified using the 8 Thrombosis LCx microparticle enzyme immunoassay: a controlled study of 5,000 patients. Am J Clin Pathol. 2007;127:68-75. doi: 10.1309/JWL-27GRGU71VP5QL.
29. Lissalde-Lavigne G, Fabbro-Peray G, Cochery-Nouvellon E, Mercier E, Ripart-Neveu S, Balducchi JP, et al. Factor V Leiden and prothrombin G20210A polymorphisms as risk factors for miscarriage during a first intended pregnancy: the matched case-control "NOHA First" study". J Thromb Haemost. 2005;3:2178-84. doi: 10.1111/j.1538-7836.2005.01581.x.
30. Gris JC, Mercier E, Qu´er´e I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M, et al. Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. Blood. 2004;103:3695-9. doi: 10.1182/blood-2003-12-4250.
31. Brenner B, Bar J, Ellis M, Yarom I, Yohai D, Samueloff A, et al. Effects of enoxaparin on late pregnancy complications and neonatal outcome in women with recurrent pregnancy loss and thrombophilia: results from the Live-Enox study. Feril Steril. 2005;84:770-3. doi: 10.1016/j.fertnstert.2005.03.048.
32. Bauersach RM; Dudenhausen J, Feridi A, Fischer T, Fung, Geisen U, et al. Risk stratification and heparin profilaxys to preven venous thromboembolism in pregnant women. Thromb Hemost. 2007;98:1237-45. doi: 10.1160/th07-05-0329.
33. Greer IA, Nelson-Piercy C. Low-molecular-weight heparins for thromboprophylaxis and treatment of venous thromboembolism in pregnancy: a systemic review of safety and efficacy. Blod. 2005;6:401-7. doi: 10.1182/blood-2005-02-0626.
34. Quenby S, Booth K, Hiller L, Coomarasamy A, de Jong PG, Hamulyák EN, et al. ALIFE2 Block Writing Committee; ALIFE2 Investigators. Heparin for women with recurrent miscarriage and inherited thrombophilia (ALIFE2): an international open-label, randomised controlled trial. Lancet. 2023 Jul 1;402(10395):54-61. doi: 10.1016/S0140-6736(23)00693-1.