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Coagulation Tests at Trigger Day in Patients with Factor V Leiden Mutation to Predict Implantation Failure

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ABSTRACT

OBJECTIVES: The aim of this study was to assess the predictive value of coagulation tests at trigger day in patients with isolated factor V Leiden mutation who underwent intracytoplasmic sperm injection cycle.

STUDY DESIGN: Ninety women with isolated factor V Leiden mutation underwent intracytoplasmic sperm injection cycles with an indication of unexplained infertility. In all participant's antagonist protocol was used for ovarian hyperstimulation and coagulation tests including activated partial thromboplastin time, partial thromboplastin time, international normalized ratio, serum fibrinogen and D-dimer levels at trigger day were determined to predict successful implantation. All the clinical parameters specific for the treatment and some patient characteristics were recorded for each participant.

RESULTS: There was no significant difference between groups with and without successful implantation in terms of age, body mass index, basal hormone levels (follicle stimulating hormone, estradiol, progesterone at day 3 and antimullerian hormone) (p>0.05). Estradiol and progesterone levels at trigger day were comparable between groups (p>0.05). Groups had similar endometrial thickness at embryo transfer day (p>0.05). Some ovarian stimulation characteristics including initial gonadotropin dose, number of follicles >17 mm, number of oocytes harvested and number of embryos were similar between groups (p>0.05). Although there was no significant difference between groups with regard to activated partial thromboplastin time and partial thromboplastin time levels, international normalized ratio level were significantly lower while D-dimer levels and fibrinogen were significantly higher in cases without implantation (p<0.05).

CONCLUSION: D-dimer, fibrinogen and international normalized ratio levels were significant predictors for successful implantation in women with isolated factor V Leiden who underwent ovulation hyperstimulation.

Keywords: Factor V leiden mutation, Thrombophilia, Embryo implantation, Clinical pregnancy

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Introduction

According to the society for assisted reproductive technology data, only 1/3 of in vitro fertilization treatments result in successful clinical pregnancy (1). In majority of the cases, early embryonal development and implantation abnormalities interfere with the progression to successful pregnancy. Implantation of blastocyst into the endometrium needs synchronized interaction between developing embryo and the endometrium which constitutes complex signaling processes and includes adhesion, nidation and trophoblastic invasion. Although these steps have not yet been clearly defined, some factors have been introduced to affect these steps. Maternal age, parity, basal hormone levels, antral follicle count, endometrial thickness and endometrial receptivity were some factors defined up to date (2,3). In addition, some studies showed that, vascular thrombosis at implantation area resulted in failure of implantation (4). During coagulation process, congenital or acquired thrombophilias were shown to be associated with abnormalities in anticoagulation and fibrinolysis cascades (5). Increased intravascular thrombin formation was shown in thrombophilias (6). In cases with thrombophilias, prothrombin is converted to thrombin and with the activation of fibrinolytic activity, fibrin degradation products especially

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the d-dimer increase in the serum (7-9). Congenital thrombophilias were shown to be associated with early pregnancy loss caused by abnormalities in vascularization during implantation (10). Factor V Leiden, prothrombin 20210G>A, methylenetetrahydrofolate reductase mutation, protein C, protein S and antithrombin 3 deficiency have been proposed to be some congenital abnormalities result in hypercoagulability (11). Factor V Leiden mutation is most commonly seen abnormality among all the congenital thrombophilias and constitutes 50 % of cases (12). Serum D-dimer and prothrombin fragments were reported to be increased in presence of deep vein or intravascular thrombosis in cases with factor V Leiden mutation (13). Thrombophilic disorders may prevent implantation, or increase early pregnancy loss and therefore have been considered to have negative impact on pregnancy outcome (10). Furthermore, according to some data, there were increased rates of congenital thrombophilias in cases of unexplained infertility (14). However, some studies reported no decreased rates in embryo implantation during assisted reproductive technology treatments in cases with factor V Leiden mutation (15). Conflicts about the association between thrombophilic disorders and implantation failure may be due to the individual differences in terms of some clinical properties of the cases.

In this study we aimed to discriminate cases with unsuccessful implantation following assisted reproduction techniques (ART) treatment by the utilization of serum D-dimer, fibrinogen, international normalized ratio (INR), activated prothrombin time (APTT) and prothrombin time (PT) in cases with factor V-Leiden mutation.

Material and Method

In this prospective cohort study, ninety consecutive nonsmoker women diagnosed to have isolated homozygous factor V Leiden mutation with no previous pregnancy or ART undergoing fresh intracytoplasmic sperm injection cycle (ICSI). Cycles at the assisted reproductive technology clinic of Zeynep Kamil Women and Children's Health Training and Research Hospital between 2015 January to March 2016 were recruited. Unexplained infertility was the indication for ICSI cycles for all participants. Unexplained infertility was defined as cases where the basic infertility workup (ovulatory cycles, normal uterine cavity, at least one patent tube on hysterosalpingography or laparoscopy, and normal semen parameters according to WHO 2010 criteria) was found to be normal. Exclusion criteria included anticoagulant use, such as heparin or aspirin, history of any hematological or any other systemic disorder, cases with cycle cancellation or absence of top quality embryo.

For all participants, a gonadotropin-releasing hormone antagonist protocol was used for ICSI. On the second day of the menstrual cycle, 150-300 IU of recombinant follicle-stimulating hormone (rFSH; Gonal-F, Merck-Serono, Geneva, Switzerland) were administered, and mean follicular growth was monitored via two-dimensional transvaginal sonography at the days determined based on follicular response. The daily dosage of rFSH was adjusted from day 5 of stimulation according to the ovarian response. The antagonist (Cetrorelix, Merck-Serono, Geneva, Switzerland) was administered at a dose of 0.25 mg/day when the follicular size reached 12-14 mm. When the follicular size reached 18 mm, 250 µcg recombinant human chorionic gonadotropin (human chorionic gonadotropin, Ovitrelle, Merck-Serono, Geneva, Switzerland) was administered subcutaneously, and follicular puncture was performed after 34-36 hours. Next, 8% vaginal progesterone gel (Crinone gel 8%, Merck-Serono, Geneva, Switzerland) was applied twice daily. ICSI was applied for each oocyte obtained by follicular puncture. Elective single grade 1 embryo (<10% fragmentation with stage-specific cell size and no multinucleation for cleavage stage grade 1 embryo and prominent, easily discernible, with many cells that are compacted and tightly adhered, many cells forming a cohesive epithelium for blastocysts) was transferred either at cleavage (day 3) or blastocyst (day 5) stage, determined by the embryo developmental characteristics. Serum levels of the Beta-hCG were measured after 2 weeks. If they were more than or equal to normal levels (10 mLU/mL) in pregnancy, the patient was considered to have successful implantation.

The clinical pregnancy was determined when ultrasound scans at 4 to 5 weeks after transfer revealed embryo with heartbeat. To analyze some mutations for hypercoagulability, DNA was extracted from blood specimens collected at the time of baseline venipuncture, before gonadotropin stimulation, and was analyzed for the presence of the mutations. Polymerase chain reaction was used to amplify a 223-bp region of the Factor V gene that encompasses nucleotide 1691. Restriction endonuclease Mnl I was used to digest the polymerase chain reaction product and generate diagnostic DNA fragments visualized after gel electrophoresis. Subjects also underwent screening for other thrombophilia causes including prothrombin and methylenetetrahydrofolate reductase gene mutation, antithrombine-3 and protein c-s deficiencies to rule coexisting thrombotic risk factor.

Clinicians managing the cycle and embryologists grading the embryos were blinded to factor V Leiden status. The primary outcome was ICSI cycle failure, or non-pregnancy, defined as a negative result on pregnancy test 14 days after transfer in a fresh ICSI cycle.

Ethical consideration: The study was approved by the institutional review board, and written consent was obtained from all subjects (27.05.2016/114).

For PT and aPTT, thromborel S (Siemens Healthcare Diagnostics, GmbH, Marburg, Germany) and Dade Actin FSL Activated PTT Reagent (Siemens Healthcare Diagnostics, GmbH, Marburg, Germany) were used as reagents, respec-

tively. For fibrinogen and d-dimer, Dade Thrombin Reagent (Siemens Healthcare Diagnostics, GmbH, Marburg, Germany) and INNO- VANCE D-dimer (Siemens Healthcare Diagnostics, GmbH, Marburg, Germany) were used as reagents. All the tests were performed using an automatic coagulation analyzer (Sysmex CA-7000, Siemens Healthcare Diagnostics, GmbH, Marburg, Germany).

Statistical analyses

Data was entered to SPSS version 15 (Chicago, USA, 2006). Continuous variables were compared by student-t test. ROC analyses was used to calculate predictive value of some variables. P < 0.05 was accepted to be statistically significant.

Results

There was no significant difference between groups with and without successful implantation in terms of age, basal hormone levels (FSH, estradiol, progesterone at day 3 and AMH) (p>0.05). Estradiol and progesterone levels at trigger day were comparable between groups (p>0.05). Groups had similar endometrial thickness at embryo transfer day (9.2 vs. 9.3 mm, p>0.05). Some ovarian stimulation characteristics including initial gonadotropin dose, number of follicles > 17 mm, number of oocytes harvested and number of embryos were similar between groups (p>0.05). Although there was no significant difference between groups with regard to APTT and PT levels, INR level were significantly lower while D-dimer and fibrinogen levels were significantly higher in cases without successful embryo implantation (p<0.05). Comparison of some demographic and clinical parameters were summarized in Table 1 and 2. INR (Area under curve=0.791, p<0.001), fibrinogen levels (AUC=0.816, p<0.001) and d-dimer (AUC=0.816, p< 0.001) levels were significant predictors for successful implantation (Figure 1 and 2). There were 4 (14.2%) miscarriages among 28 women, INR (AUC=0.766, p<0.001), fibrinogen levels (AUC=0.785, p<0.001) and d-dimer (AUC=0.785, p < 0.001) levels were significant predictors for clinical pregnancy. Implantation (4/10 vs. 24/80) and the clinical pregnancy rates (3/10 vs. 21/80) were similar between groups with cleavage stage or blastocyst transfers (p> 0.05)

Table 1: Comparison of women with and without successful embryo implantation in terms of some demographic characteristics

	IMP Negative n=62	IMP Positive n=28	*p* Value
Age (Years)	32.1±3.4	32.2±3.9	0.842
BMI (kg/m^2)	26.95±3.5	29.46±3.3	0.897
Duration of Infertility (Years)	3.8±0.9	4.1±1.1	0.164

Student-t test , IMP: Implantation, BMI: Body mass index

Table 2: Comparison of women with and without successful embryo implantation in terms of some clinical characteristics

	IMP Negative n=62	IMP Positive n=28	*p* Value
FSH (IU/L)	6.8±2.9	6.2±2.1	0.564
Estradiol* (pg/mL)	45.8±18.9	54.1±23.7	0.081
Progesterone*(ng/mL)	0.3±0.2	0.3±0.2	0.696
AMH (ng/mL)	1.5±1.7	1.7±2.2	0.683
Estradiol at trigger day (pg/mL)	1595±843.5	1746.1±847.3	0.434
Progesterone at trigger day (pg/mL)	0.5±0.2	0.5±0.2	0.9
ET at transfer(mm)	9.2±1.5	9.3±1.4	0.809
# of follicles>17 mm	8.5±3.7	9.7±3.9	0.191
# of oocytes	6.8±3.5	7.9±3.3	0.175
# of embryos	4.6±2.5	5.9±3.1	0.06
Initial gonadotropin dose	291.1±94.5	277.6±74.1	0.507
APTT(sec)	27.6±2.1	27.3±1.6	0.495
Fibrinogen (mg/dL)	370.3±60.4	297.7±62.2	<0.001
PT(sec)	12.5±1.9	12.9±0.6	0.281
Total AFC	12.7±5.1	13.1±5.3	0.744
INR	0.9±0.1	1.1±0.1	<0.001
D-Dimer (mg/dL)	3.8±0.6	3.1±0.6	<0.001

** Levels at day 3 of menstrual cycle, student-t test, IMP: Implantation, ET: Endometrial thickness, INR: international normalized ratio, APTT: activated prothrombin time, PT: prothrombin time, student-t test, N: Number, FSH: Follicle stimulating hormone, AMH: Antimullerian hormone AFC: Antral follicle coun.*

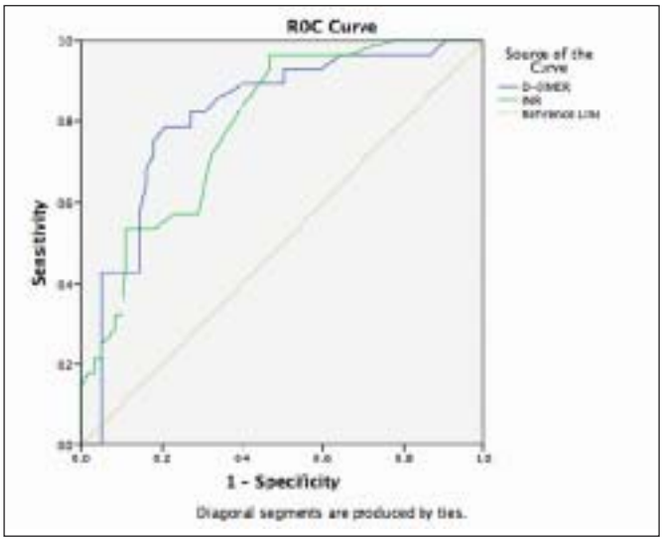

Figure 1: ROC curve of d-dimer and INR levels to predict successful embryo implantation

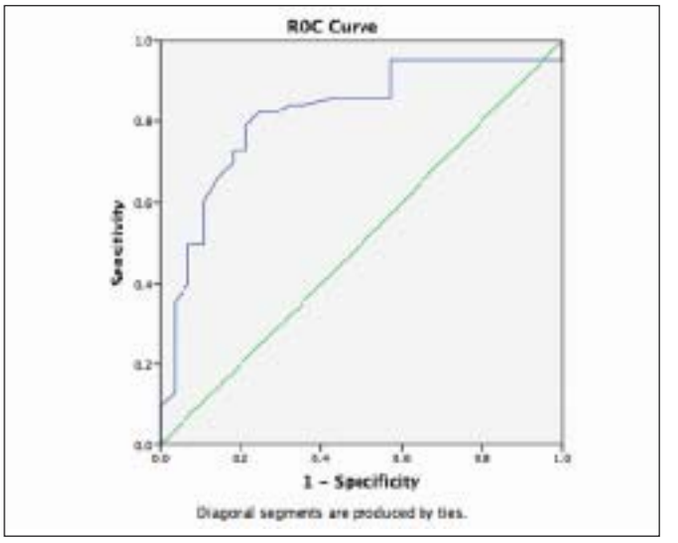

Figure 2: ROC curve of fibrinogen level to predict successful embryo implantation

Discussion

In this study, we aimed to discriminate cases with micro thrombosis secondary to elevated estrogen exposure with ovarian hyperstimulation and resulted in implantation failure following fresh cycle top quality embryo transfer in women with isolated factor V Leiden mutation. Our data revealed that assessment of some thrombotic and fibrinolytic factors may be utilized to discriminate cases with implantation failure in women with isolated thrombophilia. In cases with congenital thrombophilias, the top quality embryos are selected to be transferred to achieve pregnancy and generally embryo transfer is performed following selection of chromosomally normal embryo (16). Although 80 to 90 % of IVF cycles result in embryo transfer, among these cycles only 30-40% of cases can conceive. According to literature, there is no consensus on the main cause of failure of implantation despite the presence of optimal conditions. This high rate of failure may be attributed to the abnormalities in several steps of implantation and placentation (17). Several studies investigated the relationship between thrombophilias and embryo implantation in the literature (18). Qublan et al showed a significant association between some thrombophilias and implantation failure (19). Additionally, Azem et al found significantly higher incidence of thrombophilia among cases with failure of implantation (10). On the other hand, no difference was found between women with spontaneous pregnancy and cases apply for assisted reproduction in terms of thrombophilia rate (20). Some studies showed a significant relation specifically between factor V Leiden, prothrombin 20210 G>A and embryo implantation (18), in contrast, some data failed to show any relationship between the thrombophilias and implantation failure (15). Conflicting results coming from several studies may be due to changing inclusion and exclusion criteria of the studies or may be heterogeneity of study population. According to previous study, it was shown that 63 % of cases with genetically determined thrombophilia have combinations of different thrombophilic disorders, that may lead to heterogeneity of the study groups (21). In our study we only included the cases with isolated factor V Leiden mutations confirmed by genetical testing. According to previous studies, variable results have been reported for factor V Leiden, factor V Leiden mutation was claimed to decrease rates of implantation failure (22). On the other hand, decreased rates of implantation in cases with factor V Leiden mutation were confirmed in several studies (18). In our study protocol, we tried to show variability of response to estrogen exposure in cases with factor V Leiden mutation by the utilization of thrombotic and thrombolytic markers, however it is well known that several factors have been shown to affect embryo implantation. In our study population, most of the controllable factors that may interfere with the embryo implantation were comparable between cases with and without successful implantation. D-dimer is marker for fibrinolytic activity, levels were shown to be elevated in cases with high fibrin degradation (23). Fibrinogen was found to be decreased in cases with intravascular thrombosis due to consumption during thrombus formation (24). Results of our study led us to concentrate on possible increased rate of micro thrombosis in cases with failed implantation. In our study population, we did not observe any dyspnea, lower extremity swellings, hyperemia, diameter difference between two lower extremities, edema or tachycardia as the signs of deep vein thrombosis of lower extremities or pulmonary embolism. Although we did not see these symptoms that made us to consider about severe thrombotic events, increased thrombotic and thrombolytic markers were considered to be resulted from micro thrombotic events. Additionally, although we do not know whether it results from the ovulation induction treatment itself, it is well known that the estrogen or hormone replacement treatments

were associated with increased risk of thrombosis (25). Ovulation induction results in increased estradiol levels in the circulation which is well known thrombotic hormone. It should be considered that response to estrogen exposure may vary from subject to subject, therefore our study showed that these thrombotic and thrombolytic markers may be utilized to discriminate case with high response to estrogen exposure.

In our study, INR at trigger day were found to be shortened, d-dimer and fibrinogen levels increased in cases with failed implantation. Increased d-dimer levels showed increased thrombotic and concomitant fibrinolytic activity. Fibrinogen levels remained to be higher despite increased consumption in cases with failed implantation secondary to supra physiological estrogen levels. In the literature, authors found APTT and INR levels to be within normal levels in patients with factor V Leiden mutation (26). Instead affecting the coagulation cascade directly, factor V Leiden mutation lead to resistance against anti-fibrinolytic effect of protein C, therefore prothrombin activated prothrombin time analyses reveal within normal limits in these cases. For this reason, prolonged PT and APTT measurements in these cases may be caused by increased utilization of coagulation tests resulted from increased turnover due micro thrombotic events. Our analyses revealed same APTT and PT values in cases with and without embryo implantation. Major drawback in this study is lack of data regarding ongoing pregnancy.

D-dimer, fibrinogen and INR values with were significant predictors for successful implantation in women with isolated factor V Leiden who underwent ovulation hyperstimulation and may be used to select cases for freeze-all policy.

✍: *Conflict of interest: Authors have no conflict of interest*
Acknowledgement section: No funding source, routine tests were utilized during the study. The study was approved by the institutional review board, and written informed consent was obtained from all subjects.

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