Analysis of blood coagulation indexes, thromboelastogram and autoantibodies in patients with recurrent pregnancy loss

Huizhen Yao¹, Yimei Ji², Yanru Zhou³

ABSTRACT

Objectives: Changes in coagulation indexes, thromboelastogram(TEG) and autoantibodies in patients with recurrent pregnancy loss (RPL) with different number of abortions were analyzed.

Methods: Medical records of 48 patients with recurrent abortion, treated in Quzhou people’s Hospital from November 2019 to October 2020, were collected as the observation group. Based on the number of abortions, patients were divided into Group-A (Two abortions, n=21), Group-B (Three abortions, n=16) and group C (Abortion ≥ four times, n=11). Records of 50 healthy pregnant women in our hospital in the same period were selected as the control group. Coagulation indexes [prothrombin time (PT), activated partial prothrombin time (APTT), fibrinogen (FIB), D-Dimer (DD)], thromboelastogram (TEG) parameters [reaction time (R), coagulation time(K), maximum thrombus amplitude (MA), coagulation angle (α)], changes in the levels of autoantibodies [anticardiolipin antibody (ACA), anti-endometrial antibody (EmAb), anti-thyroid antibody(ATA)] were compared between the groups.

Results: There were significant differences in the levels of ATPP, Pt, FIB and DD among the groups. Higher number of abortions correlated with lower the levels of APTP and Pt, and higher levels of FIB and DD (P<0.05). Compared to the control group, R and K in Group-A,B and C decreased, while α and MA increased (P<0.05). There were significant differences in α and MA indexes. The positive rates of ACA, EmAb and ATA in Group-A were higher than those in the control group, but the difference was not statistically significant (P>0.05), while the above indexes in groups B and C were significantly higher than those in the control group(P<0.05). The positive rates of ACA and ATA in group C were significantly higher than those in Group-A(P<0.05), but there was no significant difference in the positive rate of EmAb (P>0.05).

Conclusion: RPL was related to the decrease of APTT, PT, and the increase of FIB and DD levels. TEG indicated that the increase of α and MA values indicated that the risk of multiple abortion was increased. The positive rates of ACA, EmAb and ATA were closely related to multiple abortions, especially the positive rates of ACA and ATA.

KEYWORDS: Anticardiolipin antibody; Antithyroid antibody, Coagulation index, Recurrent pregnancy loss, Thromboelastogram.

doi: https://doi.org/10.12669/pjms.38.7.6284

How to cite this:
Yao H, Ji Y, Zhou Y. Analysis of blood coagulation indexes, thromboelastogram and autoantibodies in patients with recurrent pregnancy loss. Pak J Med Sci. 2022;38(7):2005-2010. doi: https://doi.org/10.12669/pjms.38.7.6284

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Recurrent pregnancy loss (RPL) is a spontaneous abortion occurring more than two times and two times with the same partner. It is a common complication of obstetric pregnancy with the incidence of about 5% in women of childbearing age.¹ Some studies have pointed out that the risk of recurrent spontaneous abortion after two consecutive abortions is similar to that after three

* Received for Publication: February 18, 2022
* 1st Revision Received: March 2, 2022
* 2nd Revision Received: June 27, 2022
* Final Revision Accepted: July 6, 2022

* Correspondence: Yanru Zhou, Department of Obstetrics, Quzhou People’s Hospital, Room 601, Unit 2, Building 11, Qinqin Academy, Academy East Road, Quzhou 324000, Zhejiang Province, P.R. China. Email: yhz15167077620@163.com
consecutive abortions, suggesting that it is more reasonable to evaluate pregnancy loss after two consecutive spontaneous abortions. In women with pregnancy complications and older women it is very important to clarify the causes of spontaneous abortion as soon as possible to improve the success rate of subsequent pregnancy.2

The mechanism of RPL is complex, and in about half of cases the etiology of RPL is unknown. The common factors affecting RPL rates include heredity, autoimmune abnormalities, endocrine disorders, chromosome abnormalities, etc.3,4 The prethrombotic state has a certain impact on recurrent abortion. Hypercoagulation affects the blood flow state of the uterus and placenta, and may lead to the formation of local microthrombotic, or even placenta infarction, resulting in insufficient blood supply to the placenta, ischemia and hypoxia of the embryo or fetus, embryo- or fetal dysplasia and abortion.5 Thromboelastogram can identify the causes of abnormal coagulation function by monitoring the whole process of coagulation and fibrinolysis.6

Recent studies have suggested that more than 50% of recurrent abortions are related to immune disorder. However, the current research on this link is still limited. Additionally, there is still no consensus on the relationship between RPL and ACA, EmAb, ATA.7,8 The main goal of this study was to retrospectively explore the changes in coagulation indexes, thromboelastogram parameters and autoantibodies in RSA patients with different number of abortions to better understand a possible mechanism of RPL.

METHODS

Medical records of the 48 patients with recurrent abortion that were treated in Quzhou people’s Hospital from November 2019 to October 2020 were collected as the observation group. Based on the number of abortions, they were divided into Group-A (two abortions, n=21), Group-B (three abortions, n=16) and group C (abortion≥4 times, n=11). The control group consisted of the records of 50 healthy pregnant women with no history of infertility and recurrent abortion, who were treated in the same period in our hospital, examined by routine autoantibody tests and thromboelastogram, and had a healthy baby.

Inclusion criteria:
• Meet the RPL standard:9 Have spontaneous abortion twice or more with the same spouse, and the fetus is lost before 28 weeks of pregnancy;
• Time from last abortion ≥six months;
• Routine etiological examination results of recurrent abortion;
• There are routine results of autoantibodies and thromboelastogram.

Exclusion criteria:
• Coagulation dysfunction alone or combined with immune system diseases;
• Chromosomal abnormalities in both men and women;
• Abnormal reproductive system structure;
• The history of heart disease, diabetes, hypertension, hemorrhagic diseases and thrombotic diseases;
• Combined with malignant tumor, major organic disease, and serious infection.

The age of the women in the observation-group was 21~35 years, with an average age of 29.68±2.76 years, and that of the control group was 25~35 years, with an average age of 29.50±2.58 years, with no statistical difference between the two groups (P>0.05). This study was approved by the medical ethics committee of our institution. (Dated: April 1st, 2020).

The following relevant indexes of pregnant women during pregnancy were collected:
• PT, APTT, FIB and DD. Briefly, 5ml of fasting venous blood was collected from pregnant women in the morning of the fourth week of pregnancy into sodium citrate vacuum anticoagulant tube, fully mixed, centrifuged at a rate of 3000r/minute for 10 minute to separate plasma. The coagulation indexes were detected by Beijing pulisheng automatic hemagglutination analyzer C2000-A.
• TEG parameters of thromboelastogram, including:10 R-the time from the initial detection to the formation of the first fibrin, with a normal range of 6~8 minutes; 2) K-the time required from the end of R time to the recording amplitude of TEG analyzer for 20 minutes (normal range is three to six minutes);
• MA, the maximum amplitude of TEG, which is mainly affected by platelet aggregation function and reflects the strength of forming blood clot, with a normal range of 50~60mm;
• Solidification angle(α), the tangent of the maximum curve radian of the tracing diagram from the end of R time. The included angle between this tangent and the horizontal line is the angle, and the normal range is 50~60. Beijing Lepu thromboelastography instrument CFMS LEPU-8800 was used to detect the TEG
parameters of thromboelastography, and the operation steps were carried out according to the instructions.

- Levels of EmAb and ACA, measured by enzyme-linked immunosorbent assay (Shanghai Yuanye Biotechnology Co., Ltd) according to the manufacturer's instructions.
- ATA was detected by electrochemiluminescence using Roche’s automatic electrochemical immunoassay system and supporting reagent detection kit. Anti-thyroglobulin antibody (TgAb) ≥ 4.11iu/ml, thyroid peroxidase antibody (TPOAb) ≥ 5.61ku/ml, are considered ATA positive.

The data were analyzed by SPSS 22.0 software. The count data were expressed in (%), Fisher exact probability method was used to test. The measurement data of normal distribution were represented by ($\bar{X} \pm S$) using t-test. For one-way ANOVA, LSD-t test was used if the variance was not homogeneous. $P<0.05$ indicated that the difference was statistically significant.

**RESULTS**

There were significant differences in the levels of ATTP, Pt, FIB and DD among the groups: higher number of abortions was associated with lower levels of ATTP and Pt, and higher the levels of FIB and DD, $P<0.05$, (Table-I).

Compared with the control group, R and K in Group A, B and C decreased, while solidification angle ($\alpha$) and MA increased ($p<0.05$). There was no significant difference in R and K indexes between the groups A, B, and C ($p>0.05$), but there was significant difference in $\alpha$ and MA indexes between the three groups, $P<0.05$, (Table-II).

The positive rates of ACA, EmAb and ATA in Group-A were higher than those in the control group, but these differences were not statistically significant ($P>0.05$), while the above indexes in groups B and C were significantly higher than those

---

**Table-I: Comparison of coagulation function indexes in each group ($\bar{X} \pm S$).**

| Group               | APTT (s)   | PT (s)    | FIB (g/L) | DD (ug/L) |
|---------------------|------------|-----------|-----------|-----------|
| Control group (n=50)| 29.86±3.84 | 13.94±1.56| 3.22±0.29 | 158.26±21.30 |
| Group A (n=21)      | 26.62±3.56*| 13.05±1.96*| 3.86±0.56*| 175.90±22.39* |
| Group B (n=16)      | 24.31±2.15*| 11.88±1.63*| 4.14±0.37*| 206.25±16.01* |
| Group C (n=11)      | 21.45±2.34*| 10.55±1.86*| 4.47±0.49*| 226.45±18.89* |

| F                   | 24.404     | 15.149    | 45.754    | 45.942    |
| P                   | <0.001     | <0.001    | <0.001    | <0.001    |

**Note:** * indicates that compared with the control group, $P<0.05$; * indicates that compared with Group-A, $P<0.05$; * indicates that compared with Group-B, $P<0.05$.

**Table-II: Comparison of TEG parameters in each group ($\bar{X} \pm S$).**

| Group               | R (min)   | K (min)   | $\alpha$ (°) | MA (mm)   |
|---------------------|-----------|-----------|--------------|-----------|
| Control-group (n=50)| 3.64±0.99 | 1.16±0.31 | 78.98±1.66   | 65.40±3.74 |
| Group A (n=21)      | 3.08±1.02*| 0.87±0.23*| 82.48±1.99*  | 71.05±3.90* |
| Group B (n=16)      | 2.89±0.66*| 0.81±0.21*| 85.37±2.68*  | 74.25±3.96* |
| Group C (n=11)      | 2.67±0.59*| 0.76±0.18*| 88.45±2.30*  | 78.45±6.33* |

| F                   | 5.529     | 13.479    | 91.876      | 41.152    |
| P                   | 0.002     | <0.001    | <0.001      | <0.001    |

**Note:** * indicates that compared with the control group, $P<0.05$; * indicates that compared with Group-A, $P<0.05$; * indicates that compared with Group-B, $P<0.05$.
The positive rates of ACA and ATA in group C were significantly higher than those in Group-A ($P<0.05$), while there was no significant difference in the positive rate of EmAb ($P>0.05$), Table-III.

**DISCUSSION**

The results of our study showed that the coagulation indexes APTT and PT of RPL patients were significantly lower, while the levels of FIB and DD were significantly higher than those of healthy pregnant women ($P<0.05$). Higher number of abortions correlated with lower levels of APTT and PT, and higher levels of FIB and DD, suggesting that the risk of thrombosis in RPL patients was significantly higher than that in normal pregnant women. Studies show that increased levels of thrombosis increase the risk of RPL. Abnormal hemorheology, fibrinolysis, anticoagulation and coagulation of pregnant women during pregnancy may lead to pathological hypercoagulation, a prethrombotic state that, if continues to develop, will lead to thrombosis. Cavalcante MB et al. showed that patients with RPL had a high risk of thromboembolic events. Studies have shown that patients with RPL often suffer from placental microthrombosis, which affects the normal placental blood circulation function and fails to provide normal blood oxygen for the fetus, resulting in fetal death. Zhang K et al. pointed out that adequate uterine perfusion is very important for successful pregnancy. Since thrombosis easily leads to abnormal uterine perfusion, preventing thrombosis or improving uterine perfusion, therefore, can potentially improve pregnancy outcome and reduce the risk of abortion. Bao SH et al. studied whether the plasma DD level could guide the anticoagulation treatment of RPL associated with antiphospholipid syndrome (APS), and confirmed that women with normal DD level had the highest live birth rate. The results of our study are consistent with these reports. At the same time, studies show that RPL patients have obvious overall coagulation dysfunction that is not related to pregnancy. Therefore, clinically, detecting the coagulation indexes of RPL patients and developing corresponding treatment plans are crucial to improve the success rate of pregnancy.

Thromboelastography (TEG) has been applied in many clinical fields. Branco BC et al. used TEG to evaluate the effect of hypercoagulability in trauma patients. Shulutko EM et al. evaluated the hemostatic effect of acetaminophen based on the readings of thromboelastogram and coagulation diagram. Gordon N et al. evaluated perioperative TEG values to determine whether malignant tumor status affects blood coagulation after hepatectomy. Traditional coagulation test cannot show the process of coagulation. TEG, on the other hand, dynamically monitors the whole process of coagulation. The results of this study showed that there was no significant difference in the values of R and K among the three groups of women in the observation group. However, they were significantly lower in the observation group compared to the control group. At the same time, women in the observation group had significantly higher values of $\alpha$ and MA compared to the control group. The decrease of R and K and the increase of $\alpha$ and MA suggested that the patients in the

| Group          | ACA positive | EmAb positive | ATA positive | TgAb positive | TPOAb positive |
|----------------|--------------|---------------|--------------|---------------|----------------|
| Control-group (n=50) | 4(8%)        | 5(10%)        | 5(10%)       | 3(6%)         | 4(8%)          |
| Group A (n=21)   | 5(23.81%)    | 6(28.57%)     | 6(28.57%)    | 3(14.29%)     | 6(28.57%)      |
| Group B (n=16)   | 7(43.75%)$^a$ | 8(50.00%)$^a$ | 10(62.5%)$^a$ | 4(19.05%)     | 7(43.75%)$^a$  |
| Group C (n=11)   | 8(72.73%)$^{a,b}$ | 7(63.64%)$^a$ | 9(81.82%)$^{a,b}$ | 3(27.27%) | 9(81.82%)$^{a,b}$ |
| Fisher          | 24.408       | 19.346        | 31.28        | 6.104         | 28.537         |
| P              | <0.001       | <0.001        | <0.001       | 0.059         | <0.001         |

Note: $^*$ indicates that compared with the control group, $P<0.05$; $^a$ indicates that compared with Group-A, $P<0.05$; $^b$ indicates that compared with Group-B, $P<0.05$. In the control group ($P<0.05$). The positive rates of ACA and ATA in group C were significantly higher than those in Group-A ($P<0.05$), while there was no significant difference in the positive rate of EmAb ($P>0.05$), $P<0.05$, Table-III.
EmAb is an organ specific antibody, while ACA and ATA are non-organ specific antibodies. Studies show that EmAb may react to endometrium as an antigen, and cause a series of immune responses. Antigen-antibody reaction with endometrium may interfere with the implantation and development of fertilized eggs, resulting in abortion. However, a prospective pilot case-control study on the relationship between serum EmAb and pregnancy and abortion by Parry et al. showed that there was no significant correlation between EmAb and pregnancy and abortion. Therefore, the correlation between EmAb and recurrent abortion is still controversial. ATA includes TgAb and TPOAb. At present, the mechanism of abortion caused by ATA has not been determined, but some studies speculate that TgAb and TPOAb have a certain impact on embryo absorption and fetal immune system development, and can destroy the maternal balance during pregnancy, resulting in pathological pregnancy and even abortion. The potential mechanisms of ACA-induced abortion include developing placental circulation disorder that results in insufficient blood and oxygen supply to the fetus; placental vasculitis, resulting in insufficient fetal oxygen supply and nutrition; and thrombosis and vasoconstriction that reduce placental blood flow, and cause fetal distress and death. The results of this study also found that although the positive rates of the three antibodies in RPL patients with over four abortion were higher than those with over two abortions, only the positive rate indicators of ACA and ATA were statistically significant, while the positive rate indicators of EmAb were not statistically significant. This suggests that ACA and ATA, but not EmAb, are closely related to the number of abortions. At present, there is a lack of international research on the relationship between ACA, EmAb, ATA and specific abortion times, and further studies are needed to support our results.

Limitations of the study: 1) This is a single center study with a small sample size and relies on accurate, detailed, and available patient data due to its retrospective nature. In the future, the research design can be improved by expanding the sample population to support the conclusions of this study; Secondly, only ACA, EmAb, ATA and other auto antibody indicators are used. In the future, the relationship between auto antibody and RPL can be more comprehensively analyzed in combination with antisperm antibody (AsAb), antinuclear antibody (ANA) and other indicators; Thirdly, the coagulation indexes such as APTT, PT, FIB, DD and TEG parameters are tested separately. In the future, the test results of the two can be combined to comprehensively evaluate the coagulation state after the action of various factors of abnormal coagulation and explore its related mechanism, to provide a scientific basis for the prevention and treatment of RPL in patients who may have hypercoagulable state.

CONCLUSION

The mechanism of RPL is complex and is associated with the decrease in the levels of APTT and PT, and the increase in FIB and DD levels. TEG can better dynamically monitor the coagulation state of RPL patients. The increase of a angle and MA values correlates with the increased risk of multiple abortions. The increase in the positive rates of autoimmune antibodies ACA, EmAb and ATA is also closely related to multiple abortions, and the positive rates of ACA and ATA are closely related to the number of abortions. Therefore,
paying attention to the coagulation indexes, TEG parameters and autoimmune antibody expression in patients with RPL may improve the prognosis in RPL patients.

REFERENCES

1. Lu M, Ma F, Xiao J, Yang L, Li N, Chen D. NLRP3 inflammasome as the potential target mechanism and therapy in recurrent spontaneous abortions. Mol Med Rep. 2019;19(3):1935-1941. doi:10.3892/mmr.2019.9829
2. Dimitriadis E, Menkhort E, Saio S, Kutteh WH, Brosens JJ. Thromboprophylaxis improves the incidence and live birth rate in women with consecutive recurrent miscarriages. J Obstet Gynaecol Res. 2020;46(6):1198-1206. doi:10.1111/jog.13704
3. Yuksel H, Kayatan S, Boza AT, Api M, Ertekin AA, Cam C. Low molecular weight heparin use in unexplained recurrent miscarriage. Pak J Med Sci. 2014;30(6):1232-1237. doi:10.12669/pjms.306.5477
4. Azim M, Khan AH, Khilji ZL, Pal JA, Khurshid M. Chromosomal abnormalities as a cause of recurrent abortions: a hospital experience. J Pak Med Assoc. 2003;53(3):117-119.
5. Liu X, Chen Y, Ye C, Xing D, Wu R, Li F, et al. Hereditary thrombophilia and recurrent pregnancy loss: a systematic review and meta-analysis. Hum Reprod. 2021;36(5):1213-1229. doi:10.1093/humrep/deab101
6. Saloja N, Perry DJ. Thrombelastography [published correction appears in Blood Coagul Fibrinolysis 2001 Jan;12(1):75]. Blood Coagul Fibrinolysis. 2001;12(5):327-337. doi:10.1080/0953710700000001
7. Shetty S, Ghosh K. Anti-phospholipid antibodies and other immunological causes of recurrent foetal loss—a review of literature of various therapeutic protocols. Am J Reprod Immunol. 2009;62(1):9-24. doi:10.1111/j.1600-0897.2009.00974.x.
8. Giacomucci E, Bulletti C, Polli V, Prefetto RA, Flamigni C. Immunologically mediated abortion (IMA). J Steroid Biochem Mol Biol. 1994;49(2-3):107-121. doi:10.1016/0960-0760(94)90011-9
9. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. Curr Opin Endocrinol Diabetes Obes. 2009;16(6):446-450. doi:10.1097/MED.0b013e3283332765
10. Selby R. “TEG talk”: Expanding clinical roles for thrombelastography and rotational thromboelastometry. Hematology Am Soc Hematol Educ Program. 2020;2020(1):67-75. doi:10.1182/has.2020.00090
11. James AH. Thrombosis in pregnancy and maternal outcomes. Birth Defects Res C Embryo Today. 2015;105(5):159-166. doi:10.1002/bdr2.21106
12. Cavalcante MB, Sarno M, Cavalcante CTMB, Araujo Júnior E, Barini R. Coagulation Biomarkers in Women with Recurrent M miscarriage and Polycystic Ovarian Syndrome: Systematic Review and Meta-Analysis. Geburtshilfe Frauenheilkd. 2019;79(7):697-704. doi:10.1007/s00112-019-04114-0.
13. Carp H, Doltizky M, Inbal A. Thromboprophylaxis improves the live birth rate in women with consecutive recurrent miscarriages and hereditary thrombophilia. J Throm Haemost. 2005;3(3):433-438. doi:10.1046/j.1538-7836.2005.0066x.x
14. Zhang K, Wang E, Li Y, et al. Role of low-molecular-weight heparin in altering uterine artery blood flow in recurrent spontaneous abortion: a prospective study. J Int Med Res. 2020;48(8):300065020945558. doi:10.1177/0306049820945558
15. Bao SH, Sheng SL, Liao H, Zhou Q, Freempton ST, Tu WY. Use of D-dimer measurement to guide anticoagulant treatment in recurrent pregnancy loss associated with antiphospholipid syndrome. Am J Reprod Immunol. 2017;79(6):10.1111/ajii.12770. doi:10.1111/ajii.12770
16. Li H, Qin S, Xiao F, Li Y, Gao Y, Zhang J, et al. Predicting first-trimester outcome of embryos with cardiac activity in women with recurrent spontaneous abortion. J Int Med Res. 2020;48(6):3000650209451829. doi:10.1177/03060498209451829
17. Hao F, Zhou X, Jin L. Natural killer cells: Functional differences in recurrent spontaneous abortion. Biol Reprod. 2020;102(3):524-531. doi:10.1093/biolre/ioz201
18. Branco BC, Inaba K, Ives C, Okoye O, Shulman I, David JS, et al. Thromboelastogram evaluation of the impact of hypercoagulability in trauma patients. Shock. 2014;41(4):200-207. doi:10.1097/SHK.0000000000001019
19. Shulutko EM, Levchenko OK, Gorodetskii VM, Gemdzhan EG, Koniaishina NI, Kretchova AV. Analgesia in hemophilic patients during orthopedic surgery. Ter Arkh. 2014;86(5):56-61. Russian.
20. Gordon N, Riha G, Billingsley K, Schreiber M. Malignancy does not dictate the hypercoagulable state following liver resection. Am J Surg. 2015;209(5):870-874. doi:10.1016/j.amjsurg.2014.12.022
21. Fernández-Bello I, Stemmo C, Butta N, Lind V, Ezban M, Jiménez-Yuste V. The pharmacokinetics and pharmacodynamics of single-dose and multiple-dose recombinant activated factor VII in patients with haemophilia A or B. Haemophilia. 2017;23(6):868-876. doi:10.1111/hae.13312
22. Polokhov DM, Ershov NM, Ignatova AA, Ponomarenko EA, Gaskova MV, Zhabarkov PA, et al. Platelet function and blood coagulation system status in childhood essential thrombocythemia. Platelets. 2020;31(8):1001-1011. doi:10.1080/09537104.2019.1704710
23. Specgiornin LC, Galão EA, Bagarello LB, Oliani AH, de Godoy JM. Prevalence of anticardiolipin antibodies in pregnancies with history of repeated miscarriages. Open Rheumatol J. 2010;4:228-30. doi:10.2174/1874312901004010028.
24. Zhu H, Wang M, Dong Y, Hu H, Zhang Q, Qiao C, et al. Detection of non-criteria autoantibodies in women without apparent causes for pregnancy loss. J Clin Lab Anal. 2019;33(9):e22994. doi:10.1002/jcla.22994.
25. Bliddal S, Feldt-Rasmussen U, Rasmussen AK, Kolte AM, Hilsted LM, Christiansen OB, et al. Thyroid Peroxidase Antibodies and Prospective Live Birth Rate: A Cohort Study of Women with Recurrent Pregnancy Loss. Thyroid. 2019;29(10):1465-1474. doi:10.1089/thy.2019.0077
26. Parry AR, Calhoun BC, Ganpt P, Seybold DJ, Broce M, Randall GW. Serum anti-endometrial antibodies and first trimester pregnancy loss. W V Med J. 2013;109(6):16, 18-20.
27. Zhang J, Liu X, Cao Y. Abnormal HEM27 histone methylation of RASA1 gene leads to unexplained recurrent spontaneous abortion by regulating Ras-MAPK pathway in trophoblast cells. Mol Biol Rep. 2021;48(6):5109-5119. doi:10.1007/s11033-021-06507-6
28. Shaukat MN, Hughes P. Recurrent thrombosis and anticardiolipin antibodies associated with adenocarcinoma of the lung. Postgrad Med. 1990;66(774):316-318. doi:10.1136/pgmj.66.774.316
29. Ali S, Majid S, Niamat Ali M, Taing S, El-Serehy HA, Al-Misned FA. Evaluation of etiologic and pregnancy outcome in recurrent miscarriage patients. Saudi J Biol Sci. 2020;27(10):2809-2817. doi:10.1016/j.sjbs.2020.06.049
30. Bas FY, Tola EN, Sak S, Cankaya BA. The role of complete blood inflammation markers in the prediction of spontaneous abortion. Pak J Med Sci. 2018;34(6):1381-1385. doi:10.12669/pjms.346.13939

Authors’ Contributions:
HY: Conceived and designed the study.
HY & YJ & YZ: Collected the data and performed the analysis.
HY: Was involved in the writing of the manuscript and is responsible for the integrity of the study.
All authors have read and approved the final manuscript.

Authors:
1. Huizhen Yao
2. Yimei Ji
3. Yanru Zhou
4. Jihong Yi
5. Department of Obstetrics, Qzhou People’s Hospital, Qzhou 340000, Zhejiang Province, P.R. China.