ORIGINAL ARTICLE

The effect of low-molecular-weight heparin on live birth rate of patients with unexplained early recurrent pregnancy loss: A two-arm randomized clinical trial

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Background: The effect of anticoagulant medication in unexplained early recurrent pregnancy loss (RPL) patients is controversial. This clinical trial evaluated the effect of low-molecular-weight heparin (LMWH) on pregnancy outcomes in these patients. **Materials and Methods:** The study was performed as a single-blind randomized clinical trial between 2016 and 2018. Samples were selected from patients who were referred to Avicenna RPL clinic with a history of at least two previously happened early unexplained miscarriages. The eligibility was defined strictly to select unexplained RPL patients homogenously. One hundred and seventy-three patients who got pregnant recently were allocated randomly into two groups LMWH plus low-dose aspirin treatment (Group A = 85) and low-dose aspirin treatment only (Group B = 88)) and were followed up till their pregnancy termination (delivery/abortion). A per-protocol analysis was carried out and all statistical tests were two-sided with a $P < 0.05$ significance level. **Results:** The live birth rates (LBRs) in Groups A and B were 78% and 77.1%, respectively, which did not show any statistically significant difference between the two groups, neither in rates nor in time of abortion. In subgroup analysis for polycystic ovary syndrome (PCOS) patients, the odds ratio for study outcome (intervention/control) was 2.25 (95% confidence interval: 0.65–7.73). There was no major adverse event whereas minor bleeding was observed in 18% of patients in Group A. **Conclusion:** LMWH does not improve the LBR in unexplained RPL patients, however, it is recommended to evaluate its effect separately in PCOS patients.

Key words: Live birth rate, low-molecular-weight heparin, recurrent early pregnancy loss

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INTRODUCTION

Early miscarriage is one of the most common complications during pregnancy with a prevalence of 9%–20% in pregnant women.[1] Recurrent pregnancy loss (RPL) is a condition which traditionally defined as three or more miscarriages before the 20th week.[2] It was shown that there is no difference in the next pregnancies' miscarriage risk in patients with two or three miscarriages and therefore two miscarriages can be considered as RPL.[3-5] Based on international guidelines, medical assessment should be started after the second miscarriage.[6]

Known abnormalities such as genetic, anatomic, immunologic, endocrine, hematologic, and also male dependent factors are responsible for almost 50% of RPLs, nevertheless, the remaining called an unexplained recurrent miscarriage, has no specified known biological causes.[7] Suggested treatments for RPL with known causes are logically planned to compensate

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the pathogenic defects or relevant risk factors, hence treatment of unexplained RPL is indecisive and mainly in the experimental phase including low-dose aspirin, low-molecular-weight heparin (LMWH), progesterone, prednisolone, and intravenous immunoglobulins.[8] Most of these treatments were used successfully in RPLs with known causes, for example, thrombophilic- or immunologic-mediated RPLs.[9,10] Drug prescriptions in unexplained RPL usually are empiric and are performed based on assumptive similar causes. Thus, these interventions should be assessed precisely and separately in well-designed studies.

Many studies have been done on unexplained RPL using (LMWH) recently but the results are mostly paradoxical. Many studies showed no beneficial effect of LMWH on unexplained RPL, but on the contrary, the positive outcomes were reported rarely.[11-13] It seems patients' heterogeneity with unexplained RPL affects the studies' conclusion negatively and makes the results confusing. Most of the reviews and guidelines do not suggest LMWH injection for unexplained RPL[10,14,15] based on insufficient or nonconvincing evidence and emphasize on precise study design, large sample sizes, and elimination of confounding factors. Since Avicenna Fertility Clinic is a tertiary center for RPL, we designed a randomized clinical trial with a strict, well-defined, and uniform sample selection to study the effect of LMWH on live birth rate (LBR) of patients with unexplained early RPL.

MATERIALS AND METHODS

Design and setting

The randomized single-blind clinical trial was performed in Avicenna recurrent miscarriage clinic, Tehran, Iran, between 2016 and 2018. The study was approved by Avicenna Research Institute's Ethical Committee (No. IRACECR. Avicenna. REC1395.2) and was registered in the Iranian Registry of Clinical Trials – IRCT-numbered IRCT2016040327189N1. The aim of the study was the evaluation of LMWH prescription in unexplained RPL treatment. Since simultaneous deletion of LMWH and aspirin made patients comply and also the design of the RCT problematic, the research team decided to keep the low-dose aspirin in routine treatment of patients. Therefore, the RCT was planned in the following two arms: The LMWH and aspirin group (Group A) versus only the low-dose aspirin group (Group B).

Patient selection

All of the Iranian patients who referred to the clinic with a history of at least two or more early miscarriages (only miscarriages in the first trimester of clinical pregnancy and not molar nor biochemical pregnancies), with a normal ovarian reserve and in the age range of 18–40 years were involved in the study for more clinical assessment and getting written consent for interventions. All participants had been informed about anonymity and confidentiality during data processing and their right to withdraw from this study at any time. Patients with abnormal karyotype, any uterine abnormality (based on hysterosalpingography and ultrasound), any male factor disorders (oligospermia <2 × 106/dl, normal morphology >2%, and motility <50%), and also patients with known hereditary thrombophilia (Protein C, Protein S, and Factor V Leiden mutations, antithrombin III deficiency, and also Methylenetetrahydrofolate reductase (MTHFR) and polymorphism [PAI-1]), anti-phospholipid syndrome, and other immunologic disorders were excluded as known cause RPLs. Furthermore, the need for any assisted reproductive techniques or surgical treatments such as hysteroscopy and endometrial scratch were considered exclusion criteria either. Moreover, patients who had used heparin or other empiric treatment (e.g., prednisolone) in previous pregnancies, or with allergic reactions to heparin, also patients with anticoagulant contraindications were excluded from the sampling. In the case of thyroid dysfunction or glucose intolerance, patients underwent endocrine treatment until their tests get normal and manageable; then were included in the trial.

After a precise genetic, anatomic, and immunologic assessment, patients with normal paraclinic results such as ovarian reserve, thyroid function tests, blood sugar, antiphospholipid syndrome tests, karyotype, hereditary thrombophilia tests, MTHFR and PAI-1 gene, ultrasound and hysterosalpingography, and also spermogram were considered unexplained RPL and were asked for participation in the study.

Medical intervention

These patients were monitored for clinical pregnancy while receiving supplemental support 2 months before trying to conceive, for example, daily folic acid (5 mg, Per OS (P. O)), Vitamin B6 (40 mg P. O), selenium (200 IU P. O), Calcium D (500 mg P. O) (in cases of Vitamin D insufficiency), weekly Vitamin B12 (1000 IU, Intramuscular injection), and also 80 mg aspirin P. O. In the case of polycystic ovary syndrome (PCOS) (based on Rotterdam criteria [16]), metformin was added (1000–1500 mg P. O) to correct the blood glucose and was continued in pregnant patients with metabolic syndrome during pregnancy and discontinued in the 20th week if glucose tolerance test's result was in a normal range.[17,18] If the pregnancy had not occurred during the 1st 3 months, patients underwent a mild induction ovulation treatment. After observation of any positive Beta-human chorionic gonadotropins (β-HCG) test in the selected

patients, the patients who signed the informed consent were allocated randomly in a study group (Acetylsalicylic acid (ASA) with or without LMWH) using a computer-generated list. In a group of LMWH, a daily intramuscular injection of LMWH-CLEXAN, Sanofi Company (40 mg in prefilled syringes) was prescribed whereas the control group did not receive it. This intervention was changed to 5000 units heparin, Exir Company, Iran (subcutaneous injection twice a day) after 36th week and discontinued 24–48 h before delivery and resumed up after reduction in postpartum hemorrhage for 1 week.[19] Both groups continued aspirin, Sajad Daru, Iran, (80 mg daily P. O) medication during the pregnancy until the 32nd week but the selenium stopped. All patients were monitored throughout their pregnancy by two obstetricians in the prenatal clinic. The outcome of the clinical pregnancy (molar or only chemical pregnancy were excluded) was registered at the end of the 14th week and also at the end of the pregnancy to compare the effect of LMWH medication on pregnancy outcome. The LBR was considered the final outcome of the study. The outcome assessors and the prenatal gynecologist were blind to the study of the groups.

Sample size

Based on Shabban study[11] and using the Minitab version 17 software, assuming the 0.05 error type one, power of 80%, and the effect size of 0.2, the sample size of 85 patients in each group was calculated.

Statistical analysis

All demographic, clinical, and paraclinical information and also the outcome of the patients' pregnancy were collected and analyzed using the SPSS software version 18 (Chicago: SPSS Inc). The basic variables of the two groups were compared by Student's *t*-test, Chi-square, and Mann–Whitney (in nonparametric variables) tests. The Kaplan–Meier survival analysis and logistic regression were used to analyze the role of LMWH treatment and also other underlying factors in the improvement of ongoing pregnancy length and LBR. The level of statistical significance was defined as 5% in all of the mentioned tests.

RESULTS

Study samples

Thousand two hundred and twenty-eight RPL patients referred to the clinic in the study period. Considering the inclusion and exclusion criteria and also patients' consent, 173 informed consented patients were enrolled in random allocation [Figure 1]. Computerized random allocation dedicated 85 patients to LMWH + aspirin group and 88 patients to aspirin-only treatment. During the study, eight patients were excluded due to the following reasons: two molar pregnancies, one ectopic pregnancy (in Group A),

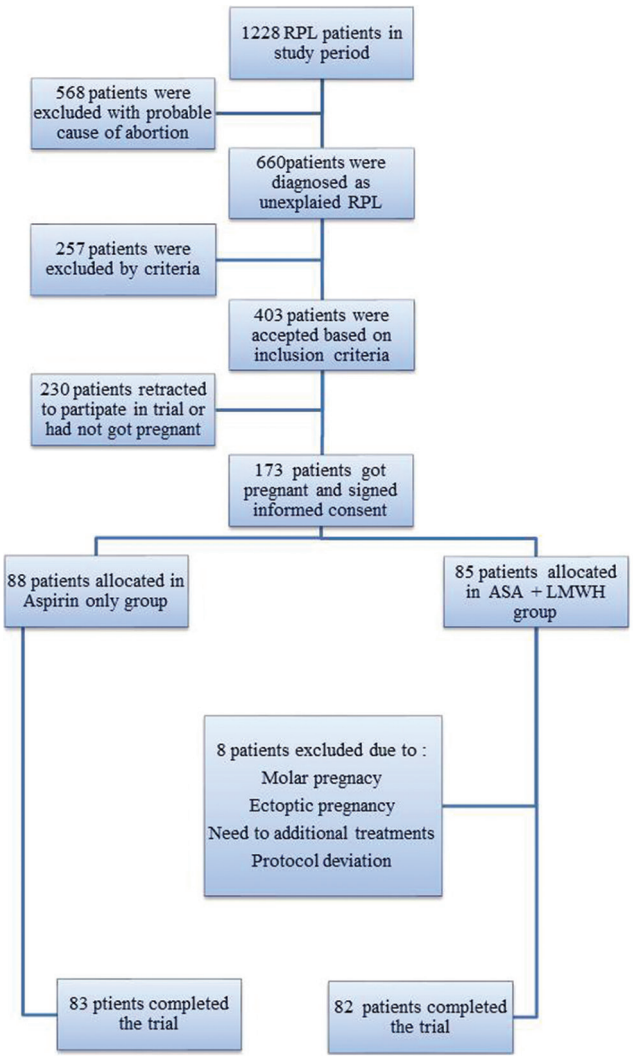

Figure 1: The CONSORT Flow Diagram of the study

three deviations from the protocol (one in Group A and two in Group B), and also two additional immunomodulatory treatments. In the end, 82 and 83 patients completed the trial in intervention and control groups, respectively [Figure 1]. The comparison of two groups of patients did not show any statistically significant difference in baseline characteristics between the two groups of patients in age, body mass index (BMI), number and time of previous abortion, serum level of follicular-stimulating hormone, and percentage of PCOS patients [Table 1].

Outcomes

All patients were followed precisely and surprisingly there was no loss to follow-up in any group (may be due to the critical situation of pregnancy in RPL patients). Patients' follow-up for pregnancy outcomes to evaluate LBR showed that the 18/82 (22%) and 19/83 (22.9%) pregnancies had lost in Groups A and B, respectively. There is no significant statistical difference between the two groups of study in

pregnancy rate (Chi-square test's $P = 0.885$). The assessment of pregnancy complications showed that three patients in Group A and one in Group B terminated their pregnancy preterm but their babies are alive which was not statistically significant (Fisher's Exact test's $P = 0.62$). In addition, one intrauterine growth restriction has occurred in Group A. The only congenital abnormality which was observed was esophageal atresia in the control group (Group B). In the LMWH group, no major bleeding accident (including intracranial hemorrhage, major gastrointestinal bleeding, hematuria, and retroplacental hematoma) occurred but 15 patients (18%) of Group A experienced at least one minor bleeding such as subcutaneous or nasal bleeding while these case were not observed merely in Group B of patients.

As the time of abortion is an important independent variable that might be affected by medical interventions, the Kaplan–Meier survival analysis has been performed to evaluate the effect of heparin injection in RPL patients. These analyses showed that there was no difference in time and the percentage of abortion between the two groups of patients (log-rank = 0.812) [Figure 2].

Multivariate analysis of pregnancy outcomes using logistic regression showed no significant relationship between pregnancy outcomes with BMI, group of intervention, and PCOS whereas the rate of ongoing pregnancy and also LBR in general (not between groups) are negatively related to age ($P = 0.054$ odds ratio [OR] = 0.87) and the number of previous miscarriage ($P = 0.001$ OR = 0.34) [Table 2]. Assessment of interaction between variables and group of interventions demonstrated that the study intervention did not change the chance of abortion considering age, BMI, and the number of previous abortions. Nevertheless, it was shown a noticeable yet nonsignificant influence of study treatment on patients suffering from PCOS ($P = 0.063$). Following subgroup analysis of PCOS patients using Chi-square test represent the nonsignificant positive effect of LMWH on pregnancy outcomes in PCOS patients either ($P = 0.193$ OR = 2.25 in non-PCOS patients and $P = 0.278$ OR = 0.595) [Table 3].

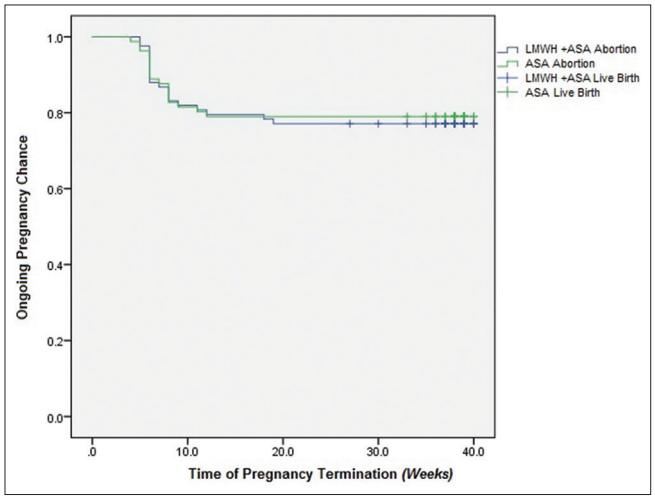

Figure 2: Kaplan Maier diagram compare the trends of pregnancies between two groups of the study

Table 1: Comparison of baseline characteristics of two groups of patients in the study

Variable	Group A (LMWH + ASA)	Group B (ASA only)	P†
Age (years)*	30.13±4.71	29.68±4.02	0.51
BMI (kg/m²)*	25.51±3.03	24.5±3.023	0.06
Time of previous abortion (weeks)**	7 (2)	6 (3)	0.08
Number of abortions***			
2	48.8 (40)	63.9 (53)	0.13
3	39 (32)	27.9 (24)	
≥4	12.2 (10)	7.2 (6)	
FSH (mIU/mL)*	7.03±2.06	6.93±95	0.79
Percentage of PCOS patients***	32.5 (27)	28 (23)	0.61

*Data have been shown as mean±SD analyzed using student *t*-test; **Data have been shown as median (IQR) and analyzed using Mann-Whitney test; ***Data have been shown as % (*n*) and analyzed using Chi-square test; †Statistical significance level was 0.05. BMI=Body mass index; FSH=Follicular stimulating hormone; PCOS=Poly cystic ovary syndrome; LMWH=Low-molecular-weight heparin; SD=Standard deviation; IQR=Interquartile range; ASA=Acetylsalicylic acid

Table 2: Multivariate analysis of predisposing factors affecting abortion chance and their interaction with the study intervention using a logistic regression model

	SE	Significance	Exp (B)	95% CI for EXP (B)
Age (years)*	0.08	0.057	0.85	0.72-1.005
LMWH medication†	4.66	0.76	0.25	0.001-2353.66
BMI (kg/m²)	0.09	0.73	1.03	0.86-1.23
PCOS*	0.64	0.019	4.5	1.28-16.37
Previous abortion*	0.31	0.001	0.47	0.18-0.62
Interactions with LMWH medication†				
Age	0.10	0.26	1.12	0.91-1.37
BMI	0.12	0.81	1.03	0.81-1.3
PCOS*	0.91	0.063	0.018	0.030-1.09
Previous abortion	0.67	0.45	0.59	0.15-2.26
Constant	3.58	0.095	396.59	

†The study intervention; *Factors which might affect the live birth rate considerably in the regression model. LMWH=Low-molecular-weight heparin; BMI=Body mass index; PCOS=Poly cystic ovary syndrome; CI=Confidence interval; SE=Standard error

Table 3: Subgroup analysis of patients' live birth based on polycystic ovary syndrome involvement in two groups of patients

PCO*	Group A (LMWH + ASA)	Group B (ASA only)	P†	95% CI	OR (for LBR)
Yes	77.8 (21)	60.9 (14)	0.19	0.65-7.73	2.25
No	76.8 (43)	84.7 (50)	0.27	0.23-1.52	0.59
Total (165)	78 (64)	77.1 (64)	0.88	0.45-1.97	0.94

†Statistical significance level was 0.05, *Data have been shown as % (*n*) and analyzed using Chi-square test. LMWH=Low-molecular-weight heparin; CI=Confidence interval; LBR=Live birth rate; OR=Odds ratio; PCOS=Poly cystic ovary syndrome ; ASA=Acetylsalicylic acid

DISCUSSION

Analyzing the main outcomes of this randomized single-blind trial showed that LMWH in combination with ASA did not improve pregnancy outcomes in patients with unexplained RPL. Importantly, a sensible positive effect was seen in patients with PCOS in subgroup and multivariate analysis; however, the lack of enough PCOS samples in the study groups reduces the power of inference and makes it inconclusive.

Although many research studies and the following meta-analysis rejected the benefits of LMWH in unexplained RPL,[20-23] it remained controversial in the field of obstetrics. At the same time, considerable RCTs report the positive effect of anticoagulant therapy yet[11,24,25] and the need for further randomized trials and individual-based meta-analysis are suggested to make a decisive conclusion.[23,26] The main obstacle in this field seems the heterogeneity of RPL patients, especially when it is unexplained. The number of previous abortions, the assumptive fetal causes for early miscarriage (before the 8th week), maternal causes for later ones, and finally the presence of underlying diseases such as PCOS complicate the situation. The current study precisely focused on patients with unexplained RPL and excluded any patients with known causes of RPL to narrow the patient's characteristics and avoid any confounding variables.

The result of this study is compatible with three large sample RCTs HABENOX,[21] ALIFE,[20] and SPIN,[22] and also a later well-designed study by Pasquier *et al*.[12] not only in the equality of pregnancy outcomes between groups of studies but also in the ranges of pregnancy rates. In addition, the calculated LBR outcome makes the result comparable with mentioned projects, whereas the results of RPL treatment in some other studies are reported as ongoing pregnancy till the 20th week or first trimester,[24] not the delivery outcomes. Nevertheless, there are some substantial differences that should be considered for interpreting. First, the type of control group treatment is placebo in some studies[12,20,21] while we prescribed ASA for the control group as minimal treatment and HABENOX study and one other performed a three groups RCT between LMWH + ASA, ASA only, and placebo group.[22,25] Second, the percentage of patients with abortion ≥3 may affect the result of interventions. The percentage of patients with more than two previous miscarriages is approximately 50% while it is a bit higher in Pasquier's study, ALIFE, and also HABENOX. Anyway, the results were similar.

It was tried to analyze data more comprehensively, thus multivariate (binary logistic) and time-dependent (Kaplan–Meier) analyses were added to the statistical method. The survival diagram illustrates that the aspirin + LMWH treatment neither improves the LBR of patients nor prolongs the time of pregnancies in cases of early abortion. This goes along with Pasquire's study results.[12]

Previous studies on PCOS patients could not prove any relationship between RPL due to thrombophilia and PCOS.[27] However, there is a study that showed patients with hyperhomocysteinemia may benefit from anticoagulant to improve the RPL.[28] In the same direction, it was shown that patients with hyperhomocysteinemia and insulin resistance who are frequently observed in PCOS patients had more chances for recurrent miscarriage.[29,30] Our results in PCOS patients suggest similar outcomes, although it was not the main question of the study and the number of PCOS patients seems too low to conclude satisfyingly. Therefore, it is suggested that these subjects to be evaluated in a randomized specified trial in PCOS patients with RPL. Since RPL is a disease with concurrent presence of inflammation and coagulation,[31] PCOS patients suffer from hyperhomocysteinemia in which leads to inflammation and autoimmunity[32] and heparin-ASA has both anticoagulant and anti-inflammatory effects,[33] this positive influence can be justified mechanistically.

Advantages and limitations

Patient selection with eligibility criteria (especially exclusion of heparin usage before the trial) made the results of the study more reliable and increased the internal validity although this led to the considerable decline of sample size and made the sampling difficult. On the other hand, the single-center performance of the trial reduced the sample size too, especially in samples with abortion history ≥ 4. Albeit the single-center study looks more homogeneous in protocol implementation, patient selection, and outcome assessment which encouraged us to do it solely.

CONCLUSION

Considering the result of the trial, it can be said that LMWH + ASA prescription in patients with unexplained repeated pregnancy loss does not change the outcomes of the patient's pregnancy. Considering the result of subgroup analysis, a specified, selected trial is suggested for the evaluation of this effect on PCOS patients with RPL.

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Conflicts of interest

There are no conflicts of interest.

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