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Effect of Enoxaparin on Live Birth Rate in Patients with Unexplained Recurrent Pregnancy Loss: A Randomized Clinical Trial

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ABSTRACT

Background & Objective: The effectiveness of aspirin and heparin in improving live birth rates in unexplained recurrent pregnancy loss remains uncertain, and further research is needed on using low molecular weight heparins (LMWH). This study was conducted with aim to assess the impact of enoxaparin, an LMWH, and its outcomes in women with URPL.

Materials & Methods: This single-blinded randomized clinical trial study was performed on 80 women with URPL and no history of thrombophilia. The participants were referred to the Infertility Clinic at Imam Reza Hospital (Milad Center) between March 2018 and February 2019. At the sixth week of gestation, the participants were assigned randomly to two groups (n=40 in each group). The treatment group received a daily subcutaneous injection of 40 mg of enoxaparin, while the control group received routine pregnancy care. Maternal and neonatal demographic data, pregnancy outcomes, complications, and live birth rates were recorded and subsequently compared between the two groups.

Results: There was no significant difference between the two groups in previous pregnancies or miscarriages, and the live birth rate was about 85% in both groups. The occurrence of pregnancy complications was significantly higher in the treatment group compared to the control group (38.2% vs. 5.7%; P=0.021).

Conclusion: Treatment with enoxaparin in women with unexplained recurrent pregnancy loss and no history of thrombophilia did not improve the pregnancy outcome nor decrease pregnancy complications.

Keywords: Recurrent Pregnancy Loss, Antiphospholipid Syndrome, Enoxaparin, Low Molecular Weight Heparin

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1. Introduction

Recurrent pregnancy loss (RPL) is a global health issue affecting approximately 2% of women of reproductive age **(1)**. RPL is defined as the occurrence of at least two spontaneous pregnancy losses before reaching a gestational age of 20 weeks **(2)**. While the cause of RPL remains elusive in approximately 50% of the cases, several risk factors have been established **(3)**. Genetic factors implicated in RPL include embryonic chromosomal aneuploidies **(4)**, maternal MTHFR C677T and paternal C677T polymorphism **(5, 6)**, and congenital uterine anomalies **(7)**. Thrombophilia has been found in 40-50% of individuals affected by RPL **(8)**, with antiphospholipid syndrome (APS) being present in 5-20% of the patients **(9)**. Although evidence suggests that genetic and immunologic factors may still play a substantial role in URPL, a subset of RPL cases remain unexplained, termed unexplained recurrent pregnancy loss (URPL) **(10, 11)**.

Now, there is no definitive management and treatment strategy for URPL. The available management strategies range from supportive care **(12)** to interventions such as intravenous immunoglobulin **(13)** and surgical management of congenital uterine anomalies **(14)**. The effectiveness of aspirin alone or in combination with heparin in improving live birth rates among women with URPL is still questionable **(15, 16)**, and the use of low molecular weight heparins

(LMWH) in reducing recurrent miscarriage requires further investigation through randomized clinical trials **(17-19)**. The current evidence is insufficient to support LMWH in URPL. Therefore, the present study was conducted with aim to evaluate the effect of enoxaparin as an LMWH and its associated outcomes in women with URPL.

2. Materials and Methods

This single-blind (data-analyst) randomized clinical trial study was performed on 80 URPL patients. The participants were recruited from women visiting the Imam Reza Hospital Infertility Clinic (Milad Center) in Mashhad between March 2018 and February 2019. Block randomization was used to assign the patients into two groups; the treatment group (n=40) received daily subcutaneous injections of enoxaparin 40 mg (Clexane®) from the 5th to 6th gestational week until the 36th week. The control group (n=40) received standard care, including regular pregnancy supplements in the same period.

The inclusion criteria were women aged 18 to 45 years with a gestational age of less than six weeks, and a history of at least two consecutive unexplained pregnancy losses. Participants with known causes or risk factors for RPL, such as systemic diseases, anatomical abnormalities, immunological disorders, endocrine dysfunctions, and chromosomal abnormalities, participants with BMI≥32 kg/m^2, and individuals who expressed unwillingness to participate at any stage or had any indication or contraindication to enoxaparin were excluded from the study.

The primary measure was the rate of live birth. Secondary outcomes included the rate of miscarriage and obstetric complications and maternal and fetal adverse outcomes such gestational diabetes, maternal hypertension and preterm birth. Data was collected using a questionnaire and observations during regular monthly follow-up visits until 37 gestational weeks.

Demographic data were presented as mean ± standard deviation or frequency and proportions as appropriate. The Chi-square and Fisher's exact test were used to compare dichotomous variables, while continuous variables were compared using the student t-test. $P < 0.05$ was considered statistically significant.

3. Results

A total of eighty eligible women were consecutively enrolled in this study and randomly assigned to either the treatment group (n=40) or the control group (n=40). The study's progression is represented in Figure 1. Importantly, no patients were excluded or lost to follow-up, resulting in a complete dataset encompassing all 80 patients.

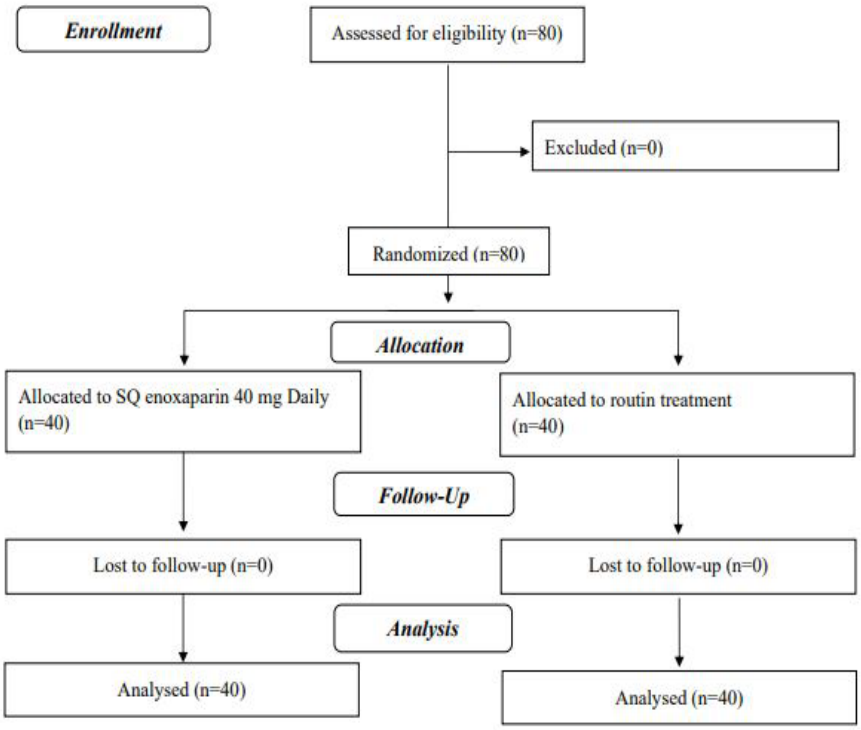


Figure 1. Flow chart of the study process

To provide a comprehensive overview of the study population, Table 1 summarizes the baseline characteristics of the participants. The mean age of participants was 32.48 ± 4.35 years ranged from 27 to

43 years. Notably, there was no significant difference between treatment and control groups in the mean age (P=0.347). Regarding the duration of marriage, the mean duration of marriage was 9.5 ± 4.42 years in the control group and 10.28 ± 5.29 years in the treatment group. There was no significant difference between the two groups in the mean duration of marriage (P=0.604). Additionally, it is worth noting that three patients in the treatment group had consanguineous marriages, while none were observed in the control group (P=0.241).

Table 1. Demographic characteristics of the participants in the treatment and control groups

Characteristics	Enoxaparin (n=40)	Control (n=40)	Total (n=80)	P-value
Age, year (mean ± SD)	32.48 ± 4.35	31.20 ± 5.15	32.48 ± 4.35	0.347
Gravida				
three	11(27.5)	3(7.5)	14(17.5)	0.103
four	13(32.5)	18(45)	31(38.75)	0.103
five	16(40)	14(35)	30(37.5)	0.103
six	0	5(12.5)	5(6.25)	0.103
History of infertility, n (%)	10 (25)	5 (12.5)	15 (18.75)	0.252
Consanguine marriage, n (%)	3(7.5)	0	3 (3.75)	0.241
Previous live birth, n (%)	24 (60)	24 (60)	48 (60)	0.290
History of low birth weight	6 (15)	10 (24)	16 (20)	0.402
Number of previous miscarriages				0.228
two	24 (60)	22 (55)	46 (57.5)	
three	13 (32.5)	13 (32.5)	26 (32.5)	
four	3 (7.5)	5 (12.5)	8 (10)	
History of preterm delivery, n (%)	12 (30)	10 (25)	22 (27.5)	0.803
History of fetal death, n (%)	18 (45)	10 (25)	28 (35)	0.100

The data is presented by number (percent) or mean± standard deviation. The Chi-square test and student t-test were applied.

There were no significant differences between the treatment and control groups in terms of gravida (P=0.103) and number of miscarriages (P=0.228).

Among the study participants, 48 patients had a history of previous live births. The live birth rate was 60% for both the enoxaparin group and the control group, with no significant difference observed between the two groups (P=0.290).

Notably, none of the patients in either the control or treatment group had a history of preeclampsia or placental abruption.

In the context of historical data regarding preterm delivery in previous pregnancies, 10 women in the control group (25%) experienced preterm delivery, while 12 cases (30%) in the treatment group also experienced the same outcome. However, this difference was not found to be statistically significant (P=0.803).

In the control group, 35 pregnancies (87.5%) resulted in live childbirth, while 34 pregnancies (85%) in the treatment group led to live births. However, this difference was not statistically significant (P=0.541).

Interestingly, three women in the enoxaparin group experienced gestational diabetes, whereas no women in the control group encountered this complication (P=0.490).

Regarding preterm birth, there was five cases of preterm birth among the women in the treatment group, while there was no preterm birth in the control group (P=0.235).

Both the control and treatment groups did not experience adverse pregnancy outcomes, such as preeclampsia, placental abruption, hemorrhage, or fetal death.

Generally, regarding pregnancy complications and adverse outcomes, the control group experienced gestational hypertension in only 2 cases. Conversely, the treatment group exhibited a broader spectrum of complications, with 3 cases of gestational diabetes, 2 cases of gestational hypertension, 3 cases of preterm labor, 2 cases of preterm premature rupture of membranes, and 3 cases of fetal growth restriction. Overall, a total of two patients (5.7%) in the control group and 13 patients (38.2%) in the treatment group encountered complications and adverse pregnancy

outcomes. A comparison between the two groups demonstrated a statistically significant difference between the two groups in the occurrence of adverse pregnancy outcomes (P=0.021).

In terms of complications such as bleeding at the injection site, bruising, or other localized reactions, there have been no reports in the treatment group and no thrombotic event was reported in the control group.

Table 2. Pregnancy outcomes and obstetrical complications of the enoxaparin and placebo groups

Outcome	Enoxaparin (n=40)	control (n=40)	P-value
Live birth, n (%)	34 (85)	35 (87.5)	0.541
Preterm delivery, n (%)	5 (12.5)	0	0.235
Low birth weight, n (%)	6 (15)	10 (24)	0.402
Gestational diabetes, n (%)	3 (7.5)	0	0.49

The data is presented by number (percent). The Chi-square test was applied.

4. Discussion

The purpose of this randomized clinical trial study was to assess the effects of enoxaparin treatment on the live birth rate of patients with URPL, comparing it to a control group receiving routine treatment. Our study findings indicate that there was no significant difference between the enoxaparin-treated group and the control group in the live birth rate. However, it is noteworthy that the incidence of complications was significantly higher in the treatment group. Therefore, based on the results of this study, enoxaparin appears to be ineffective in improving URPL when compared to the control group.

LMWH is a broadly used anticoagulant in pregnancy for the purposes of prevention and treatment of thrombotic events including venous thromboembolism (VTE) due to its proven safety and efficacy for both maternal and fetal well-being **(20, 21)**. LMWH does not cross the placenta and has fewer side effects than other anticoagulants **(22)**. Very small amounts of LMWH are detected in breast milk, indicating that it is a safe drug during breastfeeding **(23)**.

Enoxaparin as a member of the LMWHs has been the subject of several studies investigating its effect on live birth rate among patients with RPL. However, the results are conflicting, leading to a lack of consensus **(24)**.

LMWH, due to its anticoagulant and anti-inflammatory effects in combination with aspirin, was found to increase the live birth rate in PRL patients with acquired thrombophilia, APS, or constitutional thrombophilia **(25-27)**. However, the efficacy of LMWH in RPL patients even with inherited thrombophilia is still controversial. In this regard, the ALIFE and ALIFE2 studies indicated that patients with inherited thrombophilia do not benefit from LMWH combined with aspirin **(28, 29)**. Sun et al **(30)** demonstrated that patients with RPL who underwent certain assisted reproductive techniques did not derive any benefits from LMWH treatment **(30)**.

Several studies have investigated the role of LMWH in treating URPL, yielding conflicting results. Fawzy et al **(18)** in a randomized single-blind trial demonstrated that women treated with LMWH had a significantly higher birth rate. Conversely, like the present study, most of the studies indicated that LMWH fails to improve live birth rates or reduce pregnancy complications in women with URPL. In fact, when combined with low-dose aspirin, LMWH has been associated with a higher incidence of complications **(16, 17, 31, 32)**. The most common complications are bleeding, hematoma, and injection site reactions which are known side effects of enoxaparin and other low molecular-weight heparins **(33)**. Other studies did not demonstrate a significant improvement in adverse pregnancy outcomes, such as preterm delivery or intrauterine growth restrictions among women treated with LMWH **(34, 35)**. However, a noteworthy finding in the current study is that the treatment group exhibited significantly higher rates of adverse pregnancy outcomes and obstetrics complications. Heterogeneity in study designs, inclusion criteria, dosage, duration and route of LMWH treatment, and outcome measures could explain the discrepancies in the results.

In a recent systematic review and meta-analysis of eight randomized controlled studies, LMWH therapy was found to be beneficial for pregnant women with RPL. Their findings suggested that LMWH may serve as a suitable treatment option for women experiencing unexplained RPL. However, it is important to note that the small sample size of the articles and the limitations of their study require further research to validate the effectiveness and safety of LMWH **(36)**.

Limitations

This study had some limitations that should be acknowledged. The sample size was relatively small, and the study was conducted at a single center. These factors may restrict the generalizability and validity of

the results. To validate and strengthen the findings, future studies with larger sample sizes, involving multiple centers, and employing double-blind protocols are warranted.

More research needs to be conducted to identify specific subgroups of women with unexplained recurrent pregnancy loss (URPL) who may benefit from enoxaparin or other anticoagulant medications. The identification of subgroups and treatments accordingly may strengthen the effectiveness of interventions for URPL.

5. Conclusion

The present study showed no significant difference between the control and treatment groups in live birth rates. However, complications in the treatment group were significantly more prevalent compared to the control group. Thus, the administration of enoxaparin to women with a history of recurrent pregnancy loss without thrombophilia factors did not improve pregnancy outcomes or reduce pregnancy complications. Based on these findings, it is recommended that LMWH should not be used in patients with unexplained recurrent pregnancy loss (URPL) and routine care should be followed. Further research, preferably involving large multicenter randomized control trials, is needed to investigate the use of LMWH and establish uniform guidelines for treating URPL. Therefore the decision to use LMWH in URPL should be individualized and based on a careful assessment of the history and potential risks, such as prior VTE.

6. Declarations

Acknowledgments

Not applicable.

Ethical Considerations

The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences (ethical code: IR.MUMS.REC.1394.213). The study protocol was designed and conducted in accordance with the Declaration of Helsinki (2013). Informed consent was obtained from all patients prior to their enrollment.

Authors' Contributions

Seyedeh Houra Mousavi Vahed hypothesized this work. Mohadese Dashtkoohi, Mohammad Sadeq Najafi, and Mohammad Dashtkoohi contributed in the writing of the main manuscript. Nayere Khadem prepared the figures. Behnaz Ansari performed the statistical analysis. Fedyeh haghollahi and Malihe Afiat revised the manuscript. Malihe Afiat and Seyedeh Houra Mousavi Vahed designed the trial. Farbod contributed to data collection and management Seyedeh Houra Mousavi Vahed supervised the project.

Conflict of Interest

The authors declare no conflict of interest.

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