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Is enoxaparin necessary to prevent adverse pregnancy outcomes in ethylenetetrahydrofolate reductase polymorphism positive recurrent pregnancy loss cases?

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Abstract

The association between methylenetetrahydrofolate reductase polymorphism and recurrent pregnancy loss is still under debate. Moreover, the use of enoxaparin to prevent adverse pregnancy outcomes is controversial in these patients. We aimed to analyse the effect of enoxaparin on pregnancy outcomes in recurrent pregnancy loss with only methylenetetrahydrofolate reductase gene polymorphism. A total of 339 pregnant women with recurrent pregnancy loss and methylenetetrahydrofolate reductase gene polymorphism between June 2017 and March 2019 were included. Patients were divided into two groups: enoxaparin plus folic acid (n=165) and folic acid group (n=174). Then, these groups were divided into subgroups: MTHFR A1298C homozygous (n=52), MTHFR A1298C heterozygous (n=141), MTHFR C677T homozygous (n=56) and MTHFR C677T heterozygous (n=90). Pregnancy outcomes were recorded and compared between two main group, and also between subgroups. There was no significant difference between enoxaparin plus folic acid group and only folic acid group according to delivery week (p=0.287), birthweight (p=0.677), miscarriage (p=0.372), stillbirth (p=0.585), live birth (p=0.246), preterm birth (p=0.700), anomaly (p=0.883), preeclampsia (p=0.656), intrauterine growth restriction (p=0.764), neonatal intensive care unit admission (p=0.820), APGAR 1st minutes<7 (p=0.729), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=0.540). Furthermore there was no statistically significant difference with regard to delivery week, birthweight, miscarriage, stillbirth, live birth, preterm birth, anomaly, preeclampsia, intrauterine growth restriction, neonatal intensive care unit admission, APGAR 1st minute <7, APGAR 5th minute <7 and cesarean delivery between each subgroups. Contrary to the increasing trend of using empirical therapy with low molecular weight heparin in Turkey, we firstly demonstrated that there is no necessity to use enoxaparin to improve pregnancy outcomes both in homozygous and heterozygous methylenetetrahydrofolate reductase polymorphism related pregnancy loss cases. We suggest that only folic acid is enough for these cases.

Keywords: Enoxaparin, methylenetetrahydrofolate reductase, recurrent pregnancy loss, thrombophilia, habitual abortion

Introduction

Recurrent pregnancy loss (RPL), a common entity affecting approximately 0.3-1% of reproductive aged women, is defined as having two or more consecutive pregnancy losses [1]. Although the underlying pathology is idiopathic in 25-50% of all cases, several factors such as uterine malformations, chromosomal abnormalities, maternal autoimmune disorders, endocrine dysfunctions and thrombophilia have been considered for RPL etiopathogenesis [2]. In the literature, several studies have described the relationship between RPL and thrombophilia which include hereditary conditions linked to deficiencies in inhibitory coagulation factors (antithrombin III, proteins S and C) or mutations in the genes of methylenetetrahydrofolate reductase (MTHFR), prothrombin (mutation G20210A) or factor V Leiden; and acquired conditions such as antiphospholipid antibody syndrome and patients that produce lupus anticoagulants [3].

The human MTHFR gene contains 11 exons, located on chromosome 1p36.3, and encodes MTHFR which is a key enzyme in folate and homocysteine metabolism [4]. MTHFR catalyzes the biologically irreversible reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, which converts homocysteine to methionine. Some of the mutations in the MTHFR gene cause a decrement in MTHFR activity which in turn is associated with an increased risk of serebrovascular disease, venous thrombosis, neural tube defects, diabetes, cancer

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and migraine [5]. C677T and A1298C are two common forms of single nucleotide polymorphism of MTHFR gene. The reduction in enzyme activity results in hyperhomocysteinemia that is known to be related with RPL [6]. There is no consensus about the association between MTHFR polymorphism and RPL. The differences in ethnicity may be one major reason for the controversies. A meta-analysis summarizing the relationship between MTHFR C677T polymorphism and RPL has found an increased risk among carriers of the TT genotype only in a Chinese population. Furthermore, MTHFR C677T type was found to be a contributer for RPL in East Asians only. In contrast a recent meta-analysis has reported that there is no association between MTHFR A1298C polymorphism and RPL [4]. Another explainatory mechanism for the relation between MTHFR polymorphism and RPL is the homocysteine levels influenced by the MTHFR activity [7,8]. MTHFR A1298C polymorphism does not alter homocysteine levels which are tightly linked to RPL.

Another debating issue is the effect of preventive treatment with low-molecular-weight heparin (LMWH) in patients with RPL and thrombophilia. Some studies have reported an improvement in pregnancy outcomes whereas other studies have shown no useful effects of LMWH [9,10]. Moreover, a recent meta-analysis claimed that anticoagulants have not been recommended in patients with inherited thrombophilia and the prevalence of adverse pregnancy outcomes such as intrauterine growth restriction (IUGR), preeclampsia (PE), preterm delivery and congenital abnormalities were not reduced with aspirin or LMWH [11].

Contrary to these suggestions, there is an increasing trend for the use of LMWH in patients with thrombophilia and RPL in our country. One of this condition is MTHFR polymorphism. While some clinics offer only folic acid in MTHFR polymorphism positive RPL cases, others could suggest a combination theraphy of enoxaparin and folic acid in Turkey.

The aim of this study was to compare the effect of treatment with a combination of enoxaparin and folic acid versus only folic acid in MTHFR polymorphism positive RPL cases. There is only limited data in the literature searching the effect of enoxaparin in MTHFR polymorphism positive cases and there is only one study searching this issue in Turkish population but it is lack of dividing the patients into subgroups as homozygous and heterozygous. To the best of our knowledge, this is the first study searching the effect of enoxaparin in patients with only MTHFR polymorphism positive by dividing the study group into homozygous and heterozygous subgroups in Turkish population.

Materials and Methods

This is a single center, retrospective case-control study which was conducted in the department of obstetrics and gynecology of a high volume university affiliated research and training hospital between June 2017 and March 2019. Ethical approval is taken from Bursa Yuksek Ihtisas Research and Training Hospital Clinical Research Ethics Committee. All procedures performed in the study were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki Declaration.

Study Participants

A total of 339 pregnant women with recurrent RPL and MTHFR gene polymorphism between June 2017 and March 2019 were included in the study. Patients were divided into two subgroups: enoxaparin plus folic acid (n=165) and only folic acid group (n=174). Then, these groups were divided into subgroups as: MTHFR A1298C homozygous (n=52), MTHFR A1298C heterozygous (n=141), MTHFR C677T homozygous (n=56) and MTHFR C677T heterozygous (n=90). All pregnancy outcomes were compared between enoxaparin theraphy and control group in each subgroups.

Demographic characteristics of the patients, pregnancy complications, delivery mode, birth weight, APGAR scores and neonatal intensive care unit (NICU) admission were obtained from hospital medical files.

The exclusion criteria of the study were determined as follows; uterine malformations, endocrine disorders such as diabetes mellitus and thyroid disorders, thrombophilias such as factor V Leiden mutation, Protein C and S deficiencies, prothrombin gene mutation and antithrombin III deficiency, patients with infectious and autoimmune factors for RPL.

The main pregnancy outcomes of the study were determined as miscarriages, stillbirths, preterm births, congenital anomalies, PE, IUGR, cesarean section rate, birth weight, APGAR scores of neonates and NICU admission.

In our clinic, we routinely screen RPL cases for MTHFR gene polymorphism with other thrombophilias. Depending on the resident decision, in case of detecting MTHFR A1298C and C677T gene polymorphism positive, enoxaparin can be started between 6-8th week of pregnancy and continue until delivery.

MTHFR Polymorphism Analysis

Description of the screening for MTHFR gene mutation of A1298C and C677T procedure performed in our clinic was as follows: Maternal blood samples of 5 mililiter obtained from the antecubital vein were collected into tubes containing EDTA (ethylenediaminetetraacetic acid) to extract DNA. All samples were searched for MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131). Using a QIAamp DNA Blood Mini Kit (QUIAGEN, Valencia, CA), DNA was extracted. Real-time polymerase chain reaction was a technique that is used for the amplification of a DNA region. The reaction was composed of the repeating steps of heating and cooling. Bosphore MTHFR C677T and A1298C Detection Kit v1 PCR and Montania 483 (Anatolia, Turkey) were used for analysis during the study period. The procedure was applied according to the guidelines of the manufacturer.

Definiton of Pregnancy Outcomes

The following definitions were based on the current guidelines. RPL was defined as two or more consecutive spontaneous loss of a pregnancy before 22nd gestational week while pregnancy loss after 22nd week of gestation was judged as stillbirth. Preterm birth was defined as the occurence of birth before 37 weeks of gestation completed. PE was defined as the coexistance of new onset hypertension (systolic blood pressure ≥ 140/90 mmHg and/or diastolic blood pressure ≥90 mmHg) and proteinuria (0.3 gr/24 hours) or hypertension accompanied by one of the followings in

the absence of proteinuria; trombocytopenia, impairment in liver or kidney functions, cerebral dysfunction, pulmonary oedema and visual symptoms after 20th gestational week. IUGR was accepted as a birthweight below 5th percentile. Neonates were hospitalized for NICU in the presence of one of these followings; problems which need cardiac and/or respiratory monitorization, preterm infants <32nd gestational week, severe jaundice, sepsis, respiratory distress syndrome and requirement of exchange transfusion [12-14].

Statistical Analysis

Statistical analyses were carried out using SPSS statistical software version 22.0 (Statistical Package for the Social Sciences, Chicago, IL). Shapiro Wilk test was used to define whether the variables follow normal distribution. As the data of our study included non-normally distributed variables, all data were reported as median (minimum-maximum) values or in percentages. Non-normally distributed continuous variables were expressed as median while categorical variables were shown as percentage. For group comparisons, Mann-Whitney U test was used to compare continuous non-normally distributed variables and Chi-square or Fisher's exact test for categorical variables. A p value of ≤ 0.05 was accepted as statistically significant.

Results

Median age of the enoxaparin theraphy group (n=165) was 32 (15-42 years) and 30 (19-42 years) for control group (n=174) in study population. According to subgroup analysis, median age was 33 (23-42 years) for enoxaparin theraphy group and 29.5 (22-40 years) for control group in MTHFR A1298C homozygous group and 29 (20-41 years) for enoxaparin theraphy group and 33 (21-41 years) for control group in C677T homozygous group. Also, median age was 33 (15-39 years) for enoxaparin theraphy group and 29 (20-42 years) for control group in MTHFR A1298C heterozygous group. Median age was 31 (22-35 years) for enoxaparin theraphy group and 30 (19-41 years) for control group in C677T heterozygous group. There was only statistically significant with regarding to age in general population (p=0.036) and in MTHFRA1298C heterozygous group (p=0.009) between enoxaparin theraphy group and control group.

The clinical and obstetric outcomes of the patients with MTHFR polymorphism were demonstrated in Table 1. There was no statistically significant difference according to delivery week (p=0.287), birthweight (p=0.677), miscarriage (p=0.372), stillbirth (p=0.585), live birth (p=0.246), preterm birth (p=0.700), anomaly (p=0.883), PE (p= 0.656), IUGR (p=0.764), NICU admission (p=0.820), APGAR 1st minutes<7 (p=0.729), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=0.540).

The clinical and perinatal outcomes of the patients with MTHFR A1298C homozygous polymorphism were shown in Table 2. There was no statistically significant difference according to delivery week (p=0.924), birthweight (p=0.620), miscarriage (p=1.000), stillbirth (p=0.447), live birth (p=0.305), preterm birth (p=1.000), anomaly (p=0.652), PE (p= 0.690), IUGR (p=0.652), NICU admission (p=1.000), APGAR 1st minutes<7 (p=0.736), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=0.713).

The clinical and obstetric outcomes of the patients with MTHFR A1298C heterozygous polymorphism were demonstrated in Table 3. There was no statististically significant difference with regard to delivery week (p=0.574), birthweight (p=0.666), miscarriage (p=0.569), stillbirth (p=1.000), live birth (p=0.709), preterm birth (p=1.000), anomaly (p=1.000), PE (p= 1.000), IUGR (p=1.000), NICU admission (p=0.845), APGAR 1st minutes<7 (p=0.839), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=0.893).

Table 1. Pregnancy outcomes of study and control group in MTHFR polymorphism positive patients

	Enoxaparin theraphy group (n=165)	Control Group (n=174)	p
Delivery week (week)	38 (22-41)	38 (22-42)	0.287
Birth weight (gram)	3300 (650-4100)	3300 (550-4450)	0.677
Miscarriage (n, %)	27 (16.4%)	35 (20.1%)	0.372
Stillbirth (n,%)	23 (13.9%)	29 (16.7%)	0.585
Live birth (n,%)	105 (63.6%)	100 (57.5%)	0.246
Preterm Birth (n,%)	36 (21.8%)	35 (20.1%)	0.700
Anomaly (n,%)	7 (4.2%)	9 (5.2%)	0.883
Preeclampsia (n,%)	12(7.3%)	16 (9.2%)	0.656
IUGR (n,%)	10 (6.1%)	12 (7.5%)	0.764
NICU admission (n,%)	26 (15.8%)	29 (16.7%)	0.820
APGAR 1st min<7 (n,%)	20 (12.1%)	18 (10.3%)	0.729
APGAR 5th min<7 (n,%)	16 (9.7%)	17 (9.8%)	1.000
Cesarean Delivery (n,%)	67 (40.6%)	65 (37.4%)	0.540

IUGR: Intrauterin growth restriction, NICU: Neonatal intensive care unit

Table 2. Pregnancy outcomes of study and control group in MTHFR A1298c homozygous patients

	Enoxaparin theraphy group (n=28)	Control Group (n=24)	p
Delivery week (week)	38 (22-41)	38 (22-42)	0.924
Birth weight (gram)	2925 (650-3500)	3025 (550-3600)	0.620
Miscarriage (n, %)	4 (14.3%)	4 (16.7%)	1.000
Stillbirth (n,%)	3 (10.7%)	5 (20.8%)	0.447
Live birth (n,%)	19 (67.9%)	12 (50%)	0.305
Preterm Birth (n,%)	8 (28.6%)	6 (25%)	1.000
Anomaly (n,%)	2 (7.1%)	3 (12.5%)	0.652
Preeclampsia (n,%)	3 (10.7%)	4 (16.7%)	0.690
IUGR (n,%)	2 (7.1%)	3 (12.5%)	0.652
NICU admission (n,%)	5 (17.9%)	4 (16.7%)	1.000
APGAR 1st min<7 (n,%)	6 (21.4%)	4 (16.7%)	0.736
APGAR 5th min<7 (n,%)	4 (14.3%)	3 (12.5%)	1.000
Cesarean Delivery (n,%)	13 (46.4%)	9 (37.5%)	0.713

IUGR: Intrauterin growth restriction, NICU: Neonatal intensive care unit

Table 3. Pregnancy outcomes of study and control group in MTHFR A1298c heterozygous patients

	Enoxaparin theraphy group (n=67)	Control Group (n=74)	p
Delivery week (week)	38(25-41)	39(26-41)	0.574
Birth weight (gram)	3450(700-4100)	3475(850-4450)	0.666
Miscarriage (n, %)	11 (16.4%)	16 (21.6%)	0.569
Stillbirth (n,%)	11 (16.4%)	12 (16.2%)	1.000
Live birth (n,%)	41 (61.2%)	43 (58.1%)	0.709
Preterm Birth (n,%)	13 (19.4%)	15 (20.3%)	1.000
Anomaly (n,%)	2 (3%)	2 (2.7%)	1.000
Preeclampsia (n,%)	4 (6%)	5 (6.8%)	1.000
IUGR (n,%)	4 (6%)	5 (6.8%)	1.000
NICU admission (n,%)	10 (14.9%)	13 (17.6%)	0.845
APGAR 1st min<7 (n,%)	8 (11.9%)	7 (9.5%)	0.839
APGAR 5th min<7 (n,%)	6 (9%)	7 (9.5%)	1.000
Cesarean Delivery (n,%)	10 (14.9%)	13 (17.6%)	0.893

IUGR: Intrauterin growth restriction, NICU: Neonatal intensive care unit

Pregnancy outcomes of the patients with MTHFR C677T homozygous polymorphism were presented in Table 4. There was no statistically significant difference according to delivery week (p=0.723), birthweight (p=0.076), miscarriage (p=0.730), stillbirth (p=0.713), live birth (p=0.252), preterm birth (p=0.964), anomaly (p=1.000), PE (p= 0.695), IUGR (p=1.000), NICU admission (p=0.898), APGAR 1st minutes<7 (p=1.000), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=1.000).

The clinical and obstetric outcomes of the patients with MTHFR C677T heterozygous polymorphism were demonstrated in Table 5. No statistically significant difference was found according to delivery week (p=0.553), birthweight (p=0.591), miscarriage (p=1.000), stillbirth (p=1.000), live birth (p=0.949), preterm birth (p=1.000), anomaly (p=1.000), PE (p= 1.000), IUGR (p=1.000), NICU admission (p=1.000), APGAR 1st minutes<7 (p=1.000), APGAR 5th minutes<7 (p=1.000) and cesarean delivery (p=0.682).

Table 4. Pregnancy outcomes of study and control group in MTHFR C677T homozygous patients

	Enoxaparin theraphy group (n=32)	Control Group (n=24)	p
Delivery week (week)	38 (22-41)	38 (24-41)	0.723
Birth weight (gram)	3450 (650-3900)	3150 (630-3700)	0.076
Miscarriage (n, %)	5 (15.6%)	5 (20.8%)	0.730
Stillbirth (n,%)	4 (12.5%)	4 (16.7%)	0.713
Live birth (n,%)	22 (68.8%)	12 (50%)	0.252
Preterm Birth (n,%)	8 (25%)	5 (20.8%)	0.964
Anomaly (n,%)	2 (6.3%)	2 (8.3%)	1.000
Preeclampsia (n,%)	2 (6.3%)	2 (8.3%)	0.695
IUGR (n,%)	2 (6.3%)	2 (8.3%)	1.000
NICU admission (n,%)	6 (18.8%)	5 (20.8%)	0.898
APGAR 1st min<7 (n,%)	2 (6.3%)	2 (8.3%)	1.000
APGAR 5th min<7 (n,%)	2 (6.3%)	2 (8.3%)	1.000
Cesarean Delivery (n,%)	13 (40.6%)	10 (41.7%)	1.000

IUGR: Intrauterin growth restriction, NICU: Neonatal intensive care unit

Table 5. Pregnancy outcomes of study and control group in MTHFR C677T heterozygous patients

	Enoxaparin theraphy group (n=38)	Control Group (n=52)	p
Delivery week (week)	38 (26-41)	38.5 (26-41)	0.553
Birth weight (gram)	3300 (800-3850)	3300 (900-4100)	0.591
Miscarriage (n, %)	7 (18.4%)	10 (19.2%)	1.000
Stillbirth (n,%)	8 (15.4%)	8 (15.4%)	1.000
Live birth (n,%)	23 (60.5%)	33 (63.5%)	0.949
Preterm Birth (n,%)	7 (18.4%)	9 (17.3%)	1.000
Anomaly (n,%)	1 (2.6%)	2 (3.8%)	1.000
Preeclampsia (n,%)	3 (7.9%)	5 (9.6%)	1.000
IUGR (n,%)	2 (5.3%)	3 (5.8%)	1.000
NICU admission (n,%)	5 (13.2%)	7 (13.5%)	1.000
APGAR 1st min<7 (n,%)	4 (10.5%)	5 (9.6%)	1.000
APGAR 5th min<7 (n,%)	4 (10.5%)	5 (9.6%)	1.000
Cesarean Delivery (n,%)	14 (36.8%)	17 (32.7%)	0.682

IUGR: Intrauterin growth restriction, NICU: Neonatal intensive care unit

Discussion

The present study demonstrated that there is no beneficial effect of enoxaparin to improve pregnancy outcomes in RPL cases that have only heterozygous or homozygous MTHFR polymorphism in Turkish population.

RPL is one of the debating issues of obstetric practice. However RPL has been defined as three or more pregnancy losses in previous times, recent guidelines have changed this definition to two or more pregnancy losses [1,15]. Likewise the uncertanity in the definition, the association between trombophilias and RPL remain unclear. Although most large prospective studies claimed that there is no relationship between thrombophilia and pregnancy losses, case-control studies have usually reported some association [16-19]. The mechanism of these pregnancy losses is suggested to be the increased microthrombosis, infarcts and insufficiency in placental circulation due to the hemostatic impairment [20].

MTHFR is an enzyme which catalyzes the irreversible reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate. Impairment in this step results in hyperhomocysteinemia. Insufficient methylation of metabolites and toxic effect of homocysteine could be possible reasons of adverse pregnancy outcomes [21]. Hyperhomocysteinemia causes defects in chorionic villous vascularization which can lead to miscarriages and fetal growth restriction [22,23]. In the literature MTHFR gene polymorphism was found to be associated with RPL, neural tube defects and congenital anomalies in obstetric practice [24]. In a small sample sized study from Mexico demonstrated that MTHFR polymorphism is a risk factor for spontaneous abortion regardless of dietary intake of B vitamins [25].

The two most important MTHFR polymorphism consists of MTHFR C677T and MTHFR A1298C. Substitution of cytosin to timin at position 677 in MTHFR changes alanine to valin in enzyme structure and this leads to increased thermolability and disturbances in folate binding. While homozygous changes result in decrement of nearly 70% of in vitro enzyme activity, heterozygous ones affect nearly 35% of this activity. Consequently these changes cause decreased folate concentrations and elevated

homocysteine levels [26]. Likewise, in another study TT genotype of C677T polymorphism is found to be related with elevated plasma homocysteine level and increased arterial stiffness in 2008 [27]. Many studies showed that MTHFR C677T is associated with markedly increased prevalence of RPL [28-30]. Meta-analysis evaluating this relationship has found an increased RPL risk among TT genotype carriers only in Chinese subjects until 2005 [8]. In a recent meta-analysis searching the association between MTHFR C677T polymorphism and RPL supported that this polymorphism is associated with increased risk of RPL, except for Caucasians [4].

Another polymorphism in MTHFR gene is A1298C. Changing glutamate to alanine at 1298C allele decreases enzyme activity like MTHFR C677T polymorphism. This change progress better that even in homozygous ones have about only a 40% reduced activity of enzyme and it does not have higher homocysteine values as compared to controls [31,32]. Neverthless, MTHFR A1298C polymorphism may effect pregnancy outcomes due to the effects on folate metabolism [31].

Treatment is still under debate in RPL cases with MTHFR polymorphism. In current guidelines, folic acid theraphy is known to be the only recommended agent for these cases. Supporting this recommendation, in a study of Zettelberg et al, they demonstrated higher prevalence of MTHFR gene mutation in spontaneous abortion and they emphasized the protective role folic acid in these cases [24]. Furthermore, Malinow et al claimed that high plasma homocysteine levels could be lowered by folic acid supplementation in MTHFR cases [33]. Although there is no suggestion to use aspirin or LMWH in these patients in current guidelines, there are some studies focusing on this issue in the literature. In a study evaluating the effect of aspirin and LMWH in C677T MTHFR gene mutation, they found similar outcomes with normal pregnancies in 94% of all cases with aspirin and LMWH [34]. In a study from France, combination theraphy of low dose aspirin, enoxaparin and folic acid was found to be the most effective therapy in women with recurrent miscarriage who have C677T MTHFR mutation [35]. In 2014, Yuksel et al found the positive effects of LMWH on live birth in RPL cases [36]. Similarly, in 2017, Cetin et al demonstrated that live birth rates were higher in RPL patients with MTHFR polymorphism when they use enoxaparin plus folic acid [37]. LMWH was found to be preventive in placenta mediated complications in some studies in the literature [38,39]. In contrast, Fawzy et al searched for the treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage and showed that congenital anomaly rates were similar between groups who receive LMWH or not [9]. Likewise, Badawy et al did not demonstrate the effect of LMWH on congenital anomalies in early miscarriages with unknown etiology [40]. Contrary to this, Cetin et al found the rate of congenital anomaly higher in LMWH negative group in Turkish population but they concluded that this increased rate can not be depend on the usage of LMWH [37]. Badawy et al found no effect of LMWH on perinatal complications [40]. Similarly, Cetin et al showed no beneficial effect of LMWH on perinatal outcomes in MTHFR polymorphism [37]. In our study we found no difference according to the pregnancy outcomes between patients who receive LMWH or not. In addition to this, the relationship remained unsignificant in both homozygous and heterozygous treatment subgroups.

This study has several strengths. It is the first study dividing MTHFR polymorphism positive patients into groups as homozygous or heterozygous in Turkish population. Second, LMWH is standardized to enoxaparin theraphy to interprete the results more clearly by not taking into account the effects of other LMWH such as bemiparine. Last, only patients with MTHFR polymorphism (not other trombophilias such as FV Leiden, Protein C and S deficiencies) were included in the study. Contrary, it has some limitations. This study has a retrospective design with a small sample size and blood homocysteine levels were not measured.

Although there is no strong recommendation for using LMWH to prevent adverse perinatal outcomes in MTHFR polymorphism related thrombophilia among worldwide, empirical therapy with prophylactic doses of LMWH is an increasing trend in clinical practice in Turkey. Here, we firstly demonstrated that there is no necessity to use enoxaparin to improve pregnancy outcomes both in homozygous and heterozygous MTHFR polymorphism related pregnancy loss cases. We suggest that only folic acid is enough for these cases as it is claimed by international guidelines for both reducing neural tube defect and preventing adverse outcomes of MTHFR polymorhism.

Conflict of interests
The authors report no conflict of interest.

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Ethical approval
Ethical approval is taken from Bursa Yuksek Ihtisas Research and Training Hospital Clinical Research Ethics Committee.

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