Original Article

Which Protocol is better for Treatment of Ectopic Pregnancy by Methotrexate? Single-dose or Multiple-dose

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Abstract

Background: Ectopic pregnancy (EP) is the most common cause of death in the first trimester of pregnancy. Methotrexate (MTX) is an acceptable treatment in the cases with the lack of tube rupture or no important one, which has reduced surgical treatment. Despite numerous studies, there is still no consensus about medications. The present study is aimed to evaluate the single- and multiple-dose of MTX among these patients. **Materials and Methods:** This clinical trial study was done on 108 EP patients who were selected for the systemic MTX treatment and divided into two groups. For the single-dose group, MTX was administered once and β human chorionic gonadotropin (βHCG) levels were measured first and then on days 4 and 7. In the multi-dose group, 1 mg/kg MTX was injected on days 1, 3, 5, and 7. In both groups, MTX was prescribed following these days if βHCG was not reduced. In the two groups, βHCG levels were assessed after 1 week. The success rate of treatment and complications were followed up and recorded up to 6 weeks after treatment. **Results:** The success rate in the single-dose and multiple-dose MTX group was 47% and 51%. The MTX level in the single dose group decreased from 2532 ± 1154 mIU/mL to 1341 ± 553 mIU/mL and in the multiple dose group from 2671 ± 2685 mIU/mL to 1313 ± 605 mIU/mL ($P < 0.05$). Although a significant decrease was observed in each of the two groups over time, no significant difference was found between the two groups ($P > 0.05$). **Conclusion:** Single and multi-dose regimen did not show a significant difference in terms of the success of treatment. Therefore, given that the lower dose of the drug associated with lower the risk of complications, it is safe to choose the single-dose regimen.

Keywords: *Administration and dosage, ectopic pregnancy, methotrexate, treatments, treatment protocols*

Introduction

Ectopic pregnancy (EP) is the implantation of blastocyst out of uterus; 95% of EP implantation is in the fallopian tube and others in the ovary, peritoneal cavity, cervix, and scar of cesarean section. Some risk factors for EP are; prior surgery on the fallopian tube, tuboplasty after TL, sexually transmitted disease, infertility and assistant reproductive techniques, smoking and some contraception's such as intrauterine devices (IUD) and progestin's contraception's.[1] The rate of EP in the USA is about 1.3%–2% and maternal mortality due to it is about 6%, that occurs usually because of delay in diagnosis that results to perforation of the tube and active bleeding that leads to patient instability.[2] Therefore, early diagnosis and treatment of EP are very critically. Diagnosis is based on transvaginal sonography and serum β human chorionic gonadotropin (βHCG) levels.[3]

Treatment of EP can be medical or surgical. Medical treatment is the prescription of methotrexate (MTX) and surgery includes salpingostomy or salpingectomy. The type of treatment depends on the stability or instability and desire of the patient. There are several protocols for prescription of MTX in stable patients; single-, double-, multiple-dose, and injection of MTX in EP sac. MTX is an antagonist of folic acid and inhibits dihydrofolate reductase and prevents the proliferation of trophoblasts, but it has some side effects such as liver involvement, stomatitis, gastroenteritis, and bone marrow suppression. The best protocol has been a puzzle for physicians. Thus, we decided to compare the effects and side effects of single- and multiple-dose regimens in the treatment of EP.[3] The success rate of medical treatment

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has reported as between 75% and 96% in properly selected patients.[4] The multi-dose regimen includes the administration of maximum 4 dose of MTX with folinic acid.[5,6] whereas the single-dose protocol comprises a single dose of MTX, which can be repeated in nonresponders without of fulinic acid.[7]

Although both regimens have been studied extensively, there is no consensus on the optimum protocol. In a large meta-analysis of 1327 women, the success rate of single-dose was less than multi-dose regimen, without any significant difference (88% vs. 93%).[4]

Some previous studies have evaluated single-dose, two-dose, and multi-dose MTX treatment protocols for EP and have not reported a difference in therapeutic success between various doses.[8-12] In the single-dose method, after prescribing MTX at the beginning of treatment, βHCG serum levels are measured on days 4 and 7, and if no reduction of more than 15% observed, subsequent doses are prescribed.[5] However, in the two-dose or multiple-dose method, MTX is prescribed regardless of the decrease in serum βHCG level until day 4, and the other procedures are similar to the single-dose method, and surgery is recommended if there is no response to treatment and lack of appropriate reduction. Therefore, the term "single dose" seems misleading. If the initial response is not met, the patient will even receive more medication by repeating the next doses. In this regard, previous studies have rarely investigated and compared single-dose methods with two or more doses. Thus, considering that EP is one of the most important emergencies in the obstetrics and gynecology department, it has a great chance for the death of pregnant mothers and other complications. Considering that in summarizing the results of previous studies, the priority of appropriate MTX dose has not been determined, the current study attempted to examine the differences and the effect of MTX treatment as single and multiple doses and identify effective factors in the success and failure to suggest an effective regimen with the lowest dose and the least risk and complications for the patient.

Materials and Methods

The present study is a randomized clinical trial. The research population included all women with tubal EP referred to the Department of Obstetrics and Gynecology of Shahid Beheshti Hospital and Al-Zahra Hospital in 2017–2018. Based on the formula, the sample size was considered as 54 in each group. It was determined at a 95% confidence level, the test power as 80% and considering the results of previous studies concerning the success rate of single-dose and multi-dose MTX methods as 90 and 95%[13] and 5% error level. This sample was selected using nonprobability consecutive sampling from the eligible individuals who entered the study [Figure 1].

EP diagnosis was based on the serum levels of βHCG and transvaginal sonography. If the βHCG levels was >1500 mIU/mL, but there wasn't any gestational sac in the uterus or ectopic sac was seen in outside of the uterus, or increasing βHCG levels within 48 h were <66%, the diagnosis of EP was made.[14]

Inclusion criteria were the confirmation of EP by ultrasound and βHCG, stable hemodynamic status, a gestational sac with the largest diameter of 4 cm, agreement to undergo MTX treatment, and follow-up and existence of low free fluid in the abdominal and pelvic cavity based on ultrasound. In the case of active hepatitis, kidney disease, hemodynamic instability, use of immunosuppressive drugs and hematopoiesis disorder the patients were excluded.

After obtaining a code of ethics from the Ethics Committee of Isfahan University of Medical Sciences (IR. MUI. MED. REC.1397.082) and approved in the Iranian registry of the clinical trial (#IRCT20120716010297N7; https://irct. ir/trial/49305) and written consent from the eligible patient, they were randomly allocated to the single-dose and multiple-dose treatment groups. For allocation involvement, we used packet envelops and the chance for each patient to be included in one of the two groups was completely the same.

Factors such as age, gestational age, history of previous abortion, nulliparous, endometrial thickness, infertility, Use of IUD, History of surgery, use of the emergency contraceptive pill, gestational sac size of EP, and βHCG were evaluated and recorded at the beginning of the study.

In the first group, the multi-dose regimen and in the second group, the single-dose MTX regimens were administered. In the multi-dose regimen, 1 mg/kg MTX was injected on days of 1, 3, 5, and 7. It should be noted that in these days, βHCG titer was measured before MTX injection, and MTX was prescribed if there was no reduction of <15%. Leucovorin was injected at a dose of 0.1 mg/kg on days 2, 4, 6, and 8. In the single-dose regimen after MTX injection, βHCG titers were evaluated on days 4 and 7. If no reduction of 15% in βHCG titer was observed during this period, an additional dose was injected on the 7th day. The dose of MTX in the single-dose method was 50 mg/m^2 intramuscularly and did not require Leucovorin injection [Table 1].

Patients were followed for 6 weeks and the success or failure of treatment was assessed. The success of single-dose treatment means a 5% reduction in serum hCG levels after 1 week of treatment and a serum level of <5 mIU/mL after 6 weeks of treatment. In the multi-dose regimen, a 15% decrease in hCG serum levels after 48 h of treatment or after 4 therapeutic doses, or hCG serum levels <5 mIU/mL after 6 weeks of treatment was regarded as success of treatment.

In addition, if the patient has a ruptured fallopian tube during the follow-up process and requires emergency

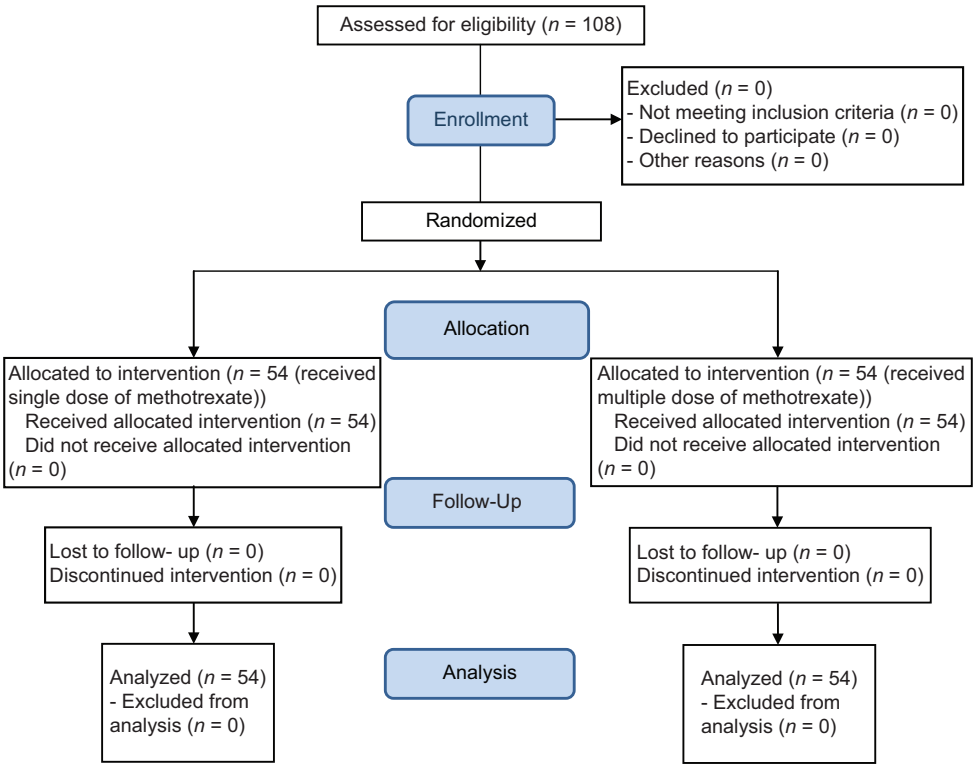

Figure 1: Consort chart

Table 1: Methotrexate regimens used to treat ectopic pregnancy

Protocol	Dose MTX	Regimen	hCG measurement	Treatment success	When to administer additional dose
Multidose	1 mg/kg and 0.1 mg/kg LEU	Alternate daily doses of each	Days 0, 1, 3, 5, 7	hCG declines 15% from previous value	2nd, 3rd or 4th dose given if hCG does not decline 15% from previous value. Maximum 4 doses
Single dose	50 mg/m^2	Day 0	Days 0, 4, 7	hCG declines 15% between day 4 and 7	Second dose on day 7 if hCG does not decline 15%

surgery, even if a decrease in serum βHCG levels occurs, it is still considered a treatment failure.

In addition, MTX complications such as hair loss, gastroenteritis, neutropenia, fever, and increase in liver enzymes were studied and recorded in the two groups during the treatment up to 6 weeks after.

Finally, the collected data was entered into SPSS software (SPSS, Inc., Chicago, IL, USA; version 22) and displayed as mean ± standard deviation or n (%). In addition, the Chi-square test was used to compare qualitative data between the two groups. The independent *t*-test was used to compare the mean quantitative variables between the two groups and the repeated measure ANOVA was used to compare the mean of the quantitative variables over time in each of the two groups. In all analyzes, the significance level was considered <0.05.

Results

The baseline characteristics of patients in the single-dose and multiple-dose groups are listed in Table 1. The mean age of the patient was similar in single-dose and multiple-dose groups (30.7 ± 5.5 vs. 29.5 ± 5.6 years). About 50% of patients had a prior history of surgery, such as cystectomy or appendectomy or tuboplasty (42.6%, 38.9%).

The use of emergency contraceptive pill and IUD in the single-dose group was reported to be 3.7% and 5.5%, respectively, and in the multiple-dose regimens, it was reported to be 3.7% and 7.4%, respectively, that was not significantly different between the two groups ($P > 0.05$) [Table 2].

On the other hand, success rate was 68.5%(37 cases) in single-dose regimen and 64.8% (35 cases) in multiple dose regimen and rate of failure was 31.5% (18.5% due to rising in βHCG levels or because of not desirable decreasing in βHCG levels and 13% for an urgent situation such as acute abdomen) and 35.2% (21% due to not desirable decreasing in βHCG levels and 14.2% for having acute abdomen). The Chi-squared test did not show a significant difference in the success rates between the single-dose and multiple-dose regimens of MTX. Furthermore, the percentage of MTX side effects was not significantly different between the two groups [Table 3].

Table 2: Baseline characteristics of patients

Characteristics	Single dose MTX	Multiple dose MTX	*P*
Age; years	30.7±5.5	29.5±5.6	0.263*
Nulliparous	20 (37.0)	17 (31.5)	0.685†
Positive history of abortion	11 (20.4)	19 (35.2)	0.247†
Infertility	11 (20.4)	9 (16.7)	0.805†
History of surgery	23 (42.6)	21 (38.9)	0.845†
Age of pregnancy; week	6.8±1.6	7.3±1.6	0.107*
Endometrial thickness; mm	8.96±4.7	8.72±4.6	0.789*
β HCG baseline; mIU/mL	2532±1154	2671±2685	0.727†
Use of IUD	3 (5.5)	2 (3.7)	0.647†
Use of emergency contraceptive pill	2 (3.7)	5 (7.4)	0.241†
Gestational sac size of ectopic pregnancy	22.2±9.5	23.7±8.5	0.389*

Data shown mean±SD or *n* (%). *Used of independent sample *t*-test, †Used of Chi-square test. SD: Standard deviation, MTX: Methotrexate, IUD: Intrauterine device, HCG: Human chorionic gonadotropin

Table 3: Treatment outcome between two groups

Outcomes	Single dose MTX (%)	Multiple dose MTX (%)	*P*†
Treatment success	37 (68.5)	35 (64.8)	0.07
Treatment failure	14 (31.5)	19 (35.2)	
Side effects*	15 (27.8)	17 (31.5)	0.64
Hair loss	4 (7.4)	5 (9.2)	0.45
Neutropenia	2 (3.7)	3 (5.5)	0.32
Fever	7 (12.9)	6 (11.1)	0.38
Increase in liver enzyme	2 (3.7)	4 (7.4)	0.21
Gastrointestinal	3 (5.5)	3 (5.5)	

*A patient may have had more than one side effect, †Used of Chi-square test. MTX: Methotrexate

The repeated measure of ANOVA used for comparison mean of βHCG levels in time trend (before, 4th and 7th injection of the first dose of MTX) between two groups.

Based on the results, over 7 days of intervention, a significant decrease in βHCG levels was observed in each of the two groups ($P = 0.03$), but no significant difference was found between the two MTX regimens ($P > 0.05$) [Table 4 and Figure 2].

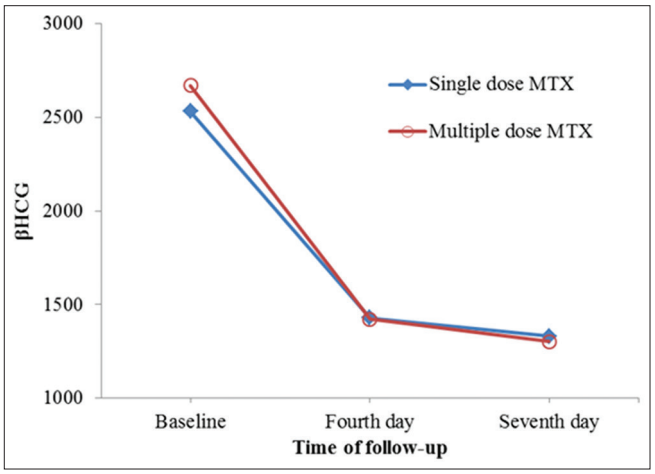

Figure 2: Mean of βHCG in two regimens at follow-up times

Discussion

With regards to MTX side effects in the treatment of EP this study was done to compare the effects of low dose versus multiple-dose. In this study, we did not find any significant differences in treatment success or side effects between single-dose and multi-dose regimens.

In our study, medical treatment by MTX was successful in 81 patients (69%) and the success rate in single-dose group was 68.5% and in the multiple-dose was 64.8%.

In a study which was done by Song *et al*.[10] The success rates between the single-dose and double-dose groups did not show a significant difference (82.6% vs. 87%).

However, the success rate in this study[10] was higher than in our study in all groups. In Song's study, some side effects were reported, and these amounts were the same as in our study. One patient exists with hair loss in multiple-dose and five patients with elevated liver enzymes.[10] In our study, no patients was not exist due to side effects.

In our study, medical treatment by MTX was successful in 81 patients (69%).

In a study by Guvendag Guven *et al*.,[13] overall success rate was in 85%. Furthermore, 80.6% of patients in single-dose regimen and 89.7% in multiple dose have a successful treatment.

Although the success rate in the present study was lower than the previous study, it should be noted that in our study, cases that were associated with tubal rupture and the need of emergency surgery despite βHCG reduction were considered as treatment failure. Therefore, this can show the accuracy and strength of this study.

In a study by Guvendag Guven *et al*.,[13] some side effects such as abdominal pain, nausea, diarrhea and elevated liver enzymes were seen in 37.5% of patients and in the single dose and multiple dose groups, 27.7% and 48.3% patients, respectively, that have been a significant differences

Table 4: Comparison of mean β human chorionic gonadotropin between the two groups at follow-up times

βHCG; mIU/mL	Single dose MTX	Multiple dose MTX	*P**
Baseline	2532±1154	2671±2685	0.727
Fourth day	1430±1523	1423±1620	0.982
Seventh day	1341±553	1313±605	0.802
*P***	*P* value of group=0.950, *P* value of time=0.03, *P* value of time × groups=0.932		

*Used of independent sample *t*-test, **Used of repeated measure ANOVA. MTX: Methotrexate, HCG: Human chorionic gonadotropin

between two groups, in our study prevalence of side effect was about 45% that was in multi-dose regimen more (48%) than single dose (41%) too, but there wasn't any significant difference between two groups.

In Balci *et al.* study[15] 294 patients with EP were treated with multiple dose MTX and the success rate of treatment in multiple dose was 78%. They concluded that multiple-dose MTX treatment had a low success rate, and the success rate was not related to initial B-HCG value; it was more related to the size of gestational mass before treatment. In our study, the mean diameter of ectopic mass was similar in two groups.

Although some studies have shown the difference between two regimens, there are some recently published meta-analysis that confirms our result:

In 2017, Yang *et al*.[16] published a systematic review and meta-analysis study for comparison of multiple-dose and double-dose versus single-dose administration of MTX for the treatment of EP.

They found six randomized controlled trials through database searching, and after the analysis, they observed that the overall success rate of multiple-dose protocol was similar to the single-dose protocol and also the difference between double-dose and single-dose groups was not significant.

About side effects, they found that the incidence of side-effects of the double-dose regimen was similar to single-dose regimen, but side-effects were more common in multiple-dose.

In our study, side effects were more common in multi-dose regimen (31.5%). Prevalence of serious side effects such as leukopenia and increase in liver enzymes was very little and was more in multi-dose regimen although these side effects were improved in follow-up period.

In addition, in the present study, complications such as hair loss, neutropenia, fever, increase in liver enzyme and gastrointestinal were observed. Although the incidence of complications in the MTX multi-dose group was higher than the single-dose group, in general, their incidence was not significantly different between the two groups.

In another systematic review and meta-analysis study that have been recently published by Yuk *et al*.[17] in the Republic of Korea, with the aim of comparison single-dose and nonsingle-dose MTX protocols in the treatment of EP have shown that single-dose and nonsingle-dose protocols had similar success rates and nonsingle-dose.

Conclusion

According to our study, there was no difference between two routs of treatment results in EP patients so we suggest single-dose regimen due to fewer serious side effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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