Advances in Bioscience and Clinical Medicine
ISSN: 2203-1413
www.abcmed.aiac.org.au

Original Paper

Methotrexate in Unruptured Ectopic Pregnancy: Comparing the Single- and Double-dose Treatment

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ARTICLE INFO

Article history
Received: March 09, 2018
Accepted: June 19, 2018
Published: July 31, 2018
Volume: 6 Issue: 3

Conflicts of interest: None
Funding: None

Key words:
Ectopic Pregnancy,
Single-dose,
Double-dose,
Methotrexate

ABSTRACT

Introduction: Ectopic pregnancy (EP) poses a great threat to pregnant women, and in case of misdiagnosis could lead to catastrophic death of the patients. EP patients tend to be treated with surgical or non-surgical treatments. One of the most common therapies in managing EP is Methotrexate (MTX), which the efficacy of its single- and double-dose treatments will be evaluated in this study. **Methods:** One-hundred and twenty patients with EP, diagnosed in AL-Zahra hospital in Tabriz, Iran, were involved in the study and were divided, randomly, into two groups of 60 (though 4 of the patients in the second group left the study due to discontent, afterwards). For the first group 50 milligrams (mg) of intramuscular (IM) single-dose MTX was prescribed, while for the second group two doses of 50 mg IM MTX were prescribed. The levels of β-hCG were evaluated before the treatment, in the fourth and seventh days, and second and fourth weeks after the treatment. In addition, sonographic findings, clinical symptoms before the treatment, and side effects after treatment were recorded. **Results:** Success rate for the single-dose group was 85%, while for the double-dose group was 94.6%. However, there was no statistically significant difference between two groups. In addition, there were no significant relations between 2 groups in endometrial thickness, presence of abdominal free fluid and gestational age with the success rate. Regardless, the initial level of β-hCG was an indicator of treatment success rate. In patients with double-dose MTX, required period of time for β-hCG levels to reduce down to zero was significantly lower than the other group. Cut-off point for success rate with single-dose MTX was 3350, with the sensitivity of 88.9% and specificity of 76.5% and for the other group it was 3894.5, with the sensitivity of 66.7% and specificity of 71.7%. **Conclusion:** No significant difference was observed between single- and double-dose MTX groups in treating EP. The initial levels of β-hCG and mass size were the only factors to predict the treatment success rate. Double-dose regimen lowered β-hCG down to zero earlier than single-dose. Based on Cut-off points, while the initial β-hCG level was below 3350, single-dose treatment was efficient, whereas, between 3350 and 3894.5, double-dose treatment was beneficial, and finally, above 3894.5, other treatments (probably surgery) were suggested.

INTRODUCTION

Ectopic pregnancy (EP) poses a great threat to pregnant women, which in case of misdiagnosis could lead to catastrophic mortality of the patients. It has been reported that up to 2% of pregnancies are ectopic or extra uterine (1). In developing countries, due to late referral to hospital, 10% of patients fail to survive (2). The rising prevalence of EP has been attributed to multiple reasons including assisted reproductive technology (ART) (3), previous history of surgery causing damages to fallopian tubes (4), contraception devices like intrauterine device (IUD) with progesterone (5), etc.

EP, mostly takes place in the uterine tube (96-98%), cervix (0.2-0.5%), - ovaries (0.2%-2%), and in less than 1% of cases, in abdominal cavity (6). Though its diagnosis used to be a big challenge in 1980's, nowadays, thanks to β-hCG levels and transvaginal sonography, most of the cases are diagnosed within early days and thus, devastating complications of the disease are prevented (7).

Two major treatment protocols exist for EP, including surgical and non-surgical treatments. Non-surgical method has approximately the same success rate as the surgical methods, while having the advantage of lower risk of infertility (8). Surgical treatments are used in cases of contra-indication and non-responders to medical treatment, hemodynamically unstable patients, and patients who do not consent for medical treatments (9). On the other hand, the most common and accepted non-surgical treatment is Methotrexate (MTX) (10, 11). MTX acts as an antagonist for folic acid, inactivates the enzyme dihydrofolate reductase, prevents the synthesis

Published by Australian International Academic Centre PTY.LTD.

http://dx.doi.org/10.7575/aiac.abcmed.v.6n.3p.1

of DNA and RNA, and it has been reported that cells which divide rapidly (like trophoblasts) are the most susceptible ones to this treatment (12).

Though, in various studies, treating with MTX has been reported to have the general efficacy of up to 95% (13-15), its side effects should be considered in treatment protocol. Some of its well-known side effects include arachnoidtis, sub-acute neurotoxocity, leukoencephalopathy, photophobia, nausea, vomiting, bone-marrow depression (dose-dependent complication), hepatotoxicity, etc. (16).

Various protocols have been suggested in this regard. Although single- and multiple-dose (including double-dose) MTX treatments have been well-studied, still there are doubts on how and when to use them (17, 18). Single-dose method consists of single shot of MTX, which in some studies is repeated once in a week for non-responding patients (19), while most common double-dose methods tend to prescribe MTX in the days 0 and 4, and monitor the levels of β-hCG in patients before and after treatment (20). β-hCG levels have been reported to be the most important factor in evaluating the failure of the MTX treatment (17, 21).

Therefore, in order to maintain the balance between the efficacy of treatment and the side-effects of MTX in EP patients, a consensus on the quality and quantity of treatments should be reached. In this study, the efficacy of single- and double-dose MTX will be evaluated. Also, the variations of β-hCG levels in response to both protocols will be reported. Finally, a cut-off point for "double-dose" treatment will be investigated.

METHODS

This is a cross-sectional clinical trial that was conducted on patients with ectopic pregnancy, referring to Alzahra hospital, Tabriz, Iran. The diagnosis was set by transvaginal sonography and serum β-hCG levels.

Sample size is calculated as below, which renders two groups of 60 (equation 1). Using method of enclosed envelope, patients were randomly assigned into two groups of single- and double-dose treatment. However, 4 patients from group 2 left the study due to dissatisfaction.

α=0/05 & β=0/2 1-β=0/8 δ=0.08 p=0/95

$$n = \frac{\left(z_{\frac{\alpha}{2}} + z_{\beta}\right)^2 p(1-p)}{\delta^2} = \frac{\left(1.96 + \frac{0.84}{}\right)^2 0.95(1-0.95)}{0.08^2} = 58$$

eq(1)

Patients' inclusion criteria included pregnant women with EP, age ranges between 15 to 50, stable hemodynamic status, gestational mass diagnosed with transvaginal sonography below 4 cm, initial levels of β-hCG below 5000 mIU/mL, and inclination to keep fertility for next pregnancies. On the other hand, exclusion criteria included Creatinine levels higher than 1.5 mg/dL, platelets amount lower than 100,000/μL, white blood cells (WBC) lower than 2000/μL, rise in hepatic enzymes more than two times of the normal ranges, presence of fetal heart beats and drug reactions

Prior to each patient's inclusion, in order to rule out any hematologic renal and hepatic diseases, related laboratory tests were obtained. For each patient a profile including age, gravidity, parity, gestational age, chief complaint, and initial levels of β-hCG, sonographic findings (i.e. presence or absence of a mass, mass size, presence of free abdominal fluid, endometrial thickness) was created. In addition, after admission, serum tests of Rh, β-hCG, complete blood count (CBC), AST, Creatinine (Cr), blood urea nitrogen (BUN) were obtained. In case of normal tests, for group one, 50 mg/m^2 of MTX in day 0, and for group two, two shots of MTX with the same dose of 50 mg/m^2 were injected in days 0 and 4. Serum β-hCG concentrations were measured and compared on days four and seven after injection. Treatment success was considered when the levels of β-hCG had a 15% reduction in comparison to prior test. Then, the levels of serum β-hCG were monitored each week until it reached 0. Based on given protocols in (12)(8), if patients' β-hCG level was not decreased down to 15%, the next dose of MTX was prescribed in day 7 and β-hCG level was checked out in day 11. If treatment failed again, another dose was administered and β-hCG levels were checked out in day 14. However, in this study it did not go further than one series of injections for both groups.

During treatment, patients were asked about their side-effects including stomach-ache and gastrointestinal signs, pain in the epigastric area, oral mucositis and hair loss.

Results including reduction in serum β-hCG levels, response to treatment and side-effects were compared and analyzed using SPSS 17. Independent t-test was used to compare the quantitative data between two groups, while for comparing qualitative data between two groups, Chi-square and Fisher's exact tests were used. For determining cut-off points ROC curve was used while sensitivity and specificity were calculated for both groups. P-value below 0.05 was considered as statistically significant.

This study was approved by the ethics committee of Tabriz University of Medical Sciences (reference number: 95/3-6/2). All patients were provided with the aims of the study, the intervention and the reason why they were chosen for the study. Also, possible side-effects of the treatment and downsides of the study were explained. They were guaranteed that their data would remain confidential and an informed consent was filled out by all the patients. All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration.

RESULTS

One hundred and twenty patients were included in this study, however, 4 of them from group two left the study due to dissatisfaction.

Age range in patients was from 20 to 45 with the mean age of 31.4±5.4, which in single-dose group was 31.1±5.2, and in the double-dose one was 31.8±5.7. There was no significant difference between two groups (p=0.45).

BMI (body mass index) range was from 21.9 to 28.3 (kg/m^2), with the mean of 25±1.2, which in the single-dose group was 24.8±2.1 and in the double-dose group was 25.2±1.1, with no significant difference between two groups (p=0.69).

The total number of patients' pregnancies was among 1 to 6 times. On the other hand, patients' total times of giving birth were among 0 to 3 times. The range of gestational age for participants in this study was 6-8 weeks. The data regarding the mean of total number of pregnancies, total number of giving births, gestational age, mean size of the masses, endometrial thickness, and admission duration are summarized in Table 1, with their P-values. Only duration of the admission had a significant difference between two groups, indicating a higher period for the double-does regimen.

The main complaints for which the patients visited the hospital were abdominal pain (81%), vaginal bleeding (11.2) and in some cases the complaint was only a delay in their menstrual cycle (7.8%).

The mean level of initial β-hCG in all patients was 3321±867, which, separately, was 3174±854 for the single-dose group and 3478±860 for the double-dose group, with no statistical significant difference (p=0.058). In addition, the relation between initial β-hCG level and response to treatment with MTX was evaluated, and showed that patients who do not respond to treatment had higher level of initial β-hCG (p=0.002).

Amongst all of the patients, the total response to MTX was 89.7%, while in the single-dose group it was 85% (51 patients) and in the double-dose group it was 94.6% (53 patients). There was no statistical significant difference between two groups (p=0.12). Surgery was required in 12 patients, where 9 patients were from single-dose group and 3 were from double-dose one, while analysis showed no significant difference (p=0.12).

After receiving the treatment, abdominal pain was reported in 15 patients; 12 from single-dose group and 3 from double-dose one. There was a significant difference between two groups, indicating higher rates of abdominal pain in single-dose regimen (p=0.02). Nevertheless, no heartburn was reported. Intestinal symptoms were observed in 5 patients; 3 from single-dose and 2 from double-dose group, which there was no statistical significant relationship between them (p=0.7). Oral mucositis happened in just one patient of single-dose group, showing no significant relation between groups (p=0.31). In addition, hair loss happened in 3 patients, 2 from single-dose and 1 from double-dose group, with no significant difference between groups (p=0.33) (Figure 1).

The β-hCG levels decline, between the 1st and 4th days, was 4±11.6%, with the range of 56.7% to -33% (- indicates increased β-hCG levels). The decline in double-dose group was significantly more than the other group (p=0.03). Also, the analysis revealed no significant relation between the decline in β-hCG levels and response to treatment (p=0.2).

Between the 1st and 7th days, the mean of β-hCG levels decline in single-dose group was 37.5±13.4 and for the double-dose group was 49.5±12.4, which in the double-dose group the decline was significantly higher (p<0.001). Between 4th and 7th days of treatment, the decline in the mean of β-hCG levels in single-dose group was 31.7±10.1 and for the double-dose group was 48.9±10.8, which, also, the decline in the double-dose group was significantly higher than the single-dose group (p<0.001).

All of the 103 patients that had their β-hCG levels checked up to 7th day, showed at least a 15% decline and all of them were responsive to the treatment.

In the single-dose group, in 16.3% of the patients, who responded to the treatment, the β-hCG levels declined to zero in the 3rd week, while in 49% of them it happened in the 4th week and in 34.7% of the cases it took more than 4 weeks. On the other hand, in the double-dose group, the decline of β-hCG levels to zero happened in the 2nd week for 7.5% of the cases, in the 3rd week for 67.9% of them, in the 4th week for 22.6% of them and for 2% of the cases it took more than 4 weeks. A statistical significant difference was observed between two groups, showing shorter duration β-hCG levels to reach down to zero in double-dose group (p<0.001).

ROC curves were implemented in order to determine the cut-off points for predicting the treatment success with single-and double-dose MTX based on β-hCG levels. Area under curve (AUC) for the single -dose regimen group was 0.838. The cut-off point for β-hCG levels to predict treatment success with single-dose regimen was 3350 with 88.9% of sensitivity and 76.5% of specificity (Figure 2).

In Addition, AUC for the double-dose regimen group was 0.786. The cut-off point for β-hCG levels to predict treatment success with double-dose regimen was 3894.5 with 66.7% of sensitivity and 71.7% of specificity (Figure 3).

DISCUSSION

This study investigated the effects of single-and double-dose MTX in treating EP and evaluated the levels of β-hCG in each treatment, trying to yield a cut-off point for each treatment based on β-hCG levels. Also, the side-effects of both

Table 1. Patients' general characteristics for two studied groups

	Total pregnancies (Mean±STD)	Total giving birth times (Mean±STD)	Gestational age* (Mean±STD)	Mass size** (Mean length±STD+Mean width±STD)	Endometrial thickness** (Mean±STD)	Admission duration*** (Mean±STD)
All patients	3±0.96	1.5±0.72	7.4±0.67	38.8±4.9+29.1±7.4	6.9±0.4	4.9±1.5
Single-dose group	2.9±1	1.4±0.72	7.2±0.74	38±5.5+28±8.5	6.9±0.4	3.7±1.2
Double-dose group	3.1±0.9	1.6±0.7	7.6±0.48	39.6±3.9+30.3±5.9	7±0.4	6±0.8
P-value	0.20	0.19	0.62	0.07	0.08	<0.001

* Weeks ** Millimeter *** Days

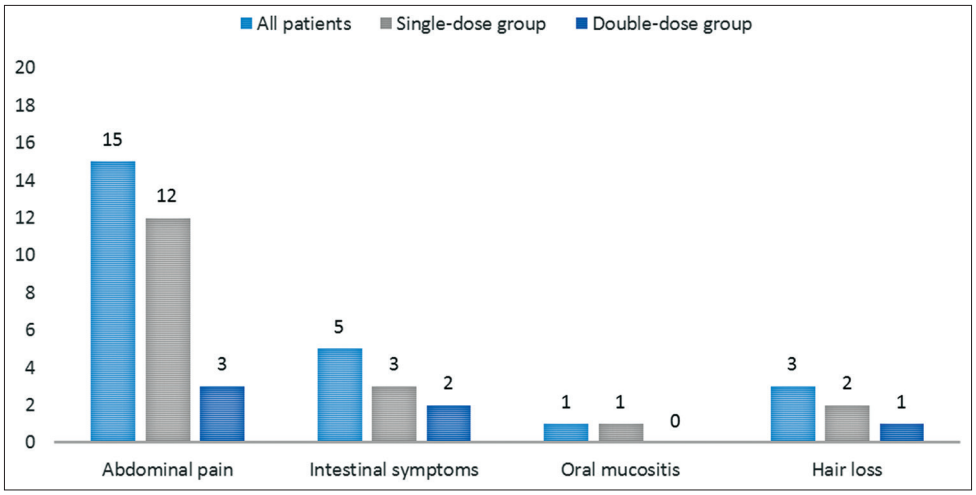

Figure 1. The frequency of side effects in two groups

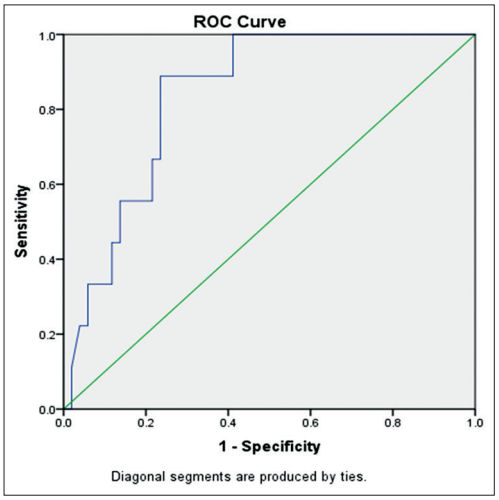

Figure 2. ROC curve for predicting treatment success with single-dose MTX based on β-hCG levels

regimens and the predictors of the treatment success were evaluated.

Multiple studies have evaluated the MTX regimen in treating EP. In a study by Tas et al. in 2017 (22), effects of single-dose MTX was evaluated in EP and the treatment success rate was 72.4% and mostly, it showed efficacy in patients who had lower initial β-hCG levels. They assessed the success cut-off point of treatment based on β-hCG levels around 2678 IU/L, with 75% sensitivity and 73.8% specificity, whereas initial β-hCG levels below 2678 IU/L, had 8.45 times of more failures in treatment. Also, they reported that fetal cardiac activity raised the treatment failure up to 12 times, while history of EP, endometrial thickness, progesterone value and free pelvic fluid had no effects on treatment failure. However in this study the treatment success using single-dose of MTX was reported to be 85%, showing higher rates of treatment success than Tas's study. On the other hand, the cut-off point for single-dose MTX was 3350 IU/L, which was also higher. Nevertheless, similar to Tas's study we found no relation between treatment success and thickness of endometrium, and pelvic free fluid (22).

Kim et al. in 2017 (23) evaluated the β-hCG levels cut-off points for single- and multi-dose MTX treatments in EP patients and reported a single-dose treatment success of 64.2% and multi-dose success of 71.9%. Similar to our study, initial β-hCG test was introduces as an independent prognostic factor in predicting treatment success. On the other hand, cut-off points for single-dose group and multi-dose group were 3026 IU/L and 3711 IU/L, respectively.

Bonin et al. (24) depicted an overall treatment success rate of 78.5% with MTX, while, a 63.5% success with single-dose MTX and a 73.2% success with double-dose MTX was reported. Similar to our study and many other studies initial β-hCG levels was of importance in predicting treatment success, with 90% of successful treatment in patients who had pretreatment β-hCG levels<1000.

However, in a study by Jurkovic et al. (25), using MTX (83% of treatment success) showed no statistically significant advantage over placebo (76% of treatment success) in treating EP. In addition, in a systematic review by Yang et al. (26), the treatment success for single- and multi-dose groups in various studies showed no significant relation. Also, this study showed similar prevalence of side-effects in single- and double-dose treatment regimens, while a significant higher prevalence of side-effects in multi-dose treatments was reported. They concluded that double-dose MTX is a safe treatment for EP. Similar to this review, we also, found no significant difference between treatment success and side-effects in both regimens, except for abdominal pain, that was higher in single-dose group in our study. Better responses to double-dose MTX might be the reason to lower abdominal pain in patients treated with double dose MTX.

Song et al. (27) reported that there are no significant differences between treatment success, side-effects, costs and satisfaction from the treatment with regimen type. However, double-dose treatment, like our study, was shown to reduce the time period required for β-hCG levels to fall down to zero.

In another study by Mirbolouk and colleagues in 2015 (28), single-dose treatment with MTX showed success in 77.1% of the patients, while pretreatment β-hCG level and its reduction in 4th day were illustrated to be the best predic-

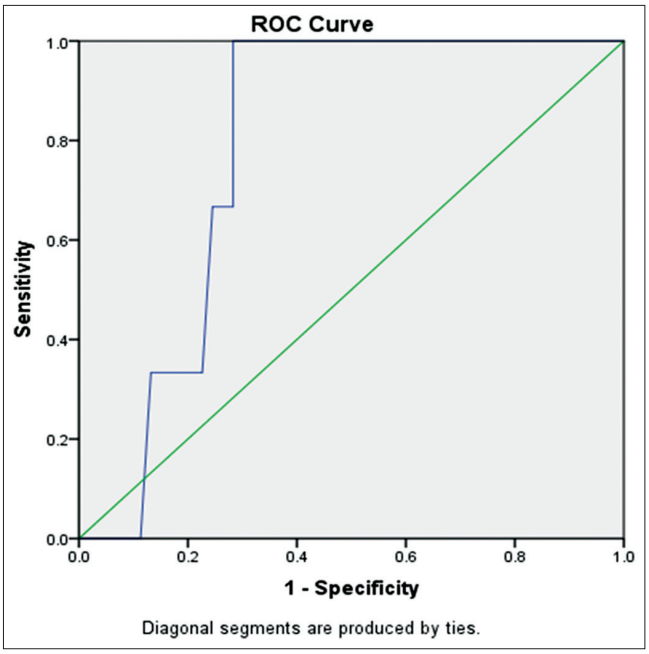

Figure 3. ROC curve for predicting treatment success with double-dose MTX based on β-hCG levels

tors of treatment success. Also, cut-off point for single-dose MTX was set to be 1375 IU/L. However, in our study no significant relation was obtained between β-hCG levels decline between days 1 and 4, and cut-off point for single-dose regimen was higher than this study.

Wu and colleagues (29) in 2014, reported that cut-off point for β-hCG level with single-dose treatment of MTX was 1202 IU/L with specificity of 74% and sensitivity of 84%. The treatment success was reported to be 84% which was close to ours. Side-effects included nausea, abdominal pain, vaginal bleeding, however, serious complications were reported to be rare, same as our study.

Besides, Avcıuoglu et al. in 2014 (30) reported no significant difference between single-dose (70.14% of treatment success) and double-dose (70%) regimen. They also depicted that in β-hCG levels<1000 IU/L, treatment success rises up to 86.11%, while in β-hCG levels>3000 IU/L treatment success was limited to 42.3%. Similar to our study, there was a significant relation between mass size and treatment success, i.e. with the mass size more than 25mm there was only 89.28% of success while for the sizes below 25mm it was 89.28%.

In another study conducted by Skubisz et al. (31), 89% of patients showed 15% reduction in β-hCG levels between 4th and 7th days in response to single-dose MTX, while it was 85% for the 1st to 4th days. However, our study showed that 100% of participants had at least 15% of decreased β-hCG level within 4th to 7th days.

CONCLUSION

The results of this study depict that, though treating EP with double-dose regimen of MTX was efficient in comparison to single-dose regimen, the difference was not significant. Initial levels of β-hCG and mass size were the only factors that predicted the treatment success correctly. The only significant difference in side-effects was observed in the abdominal pain, where it was higher in single-dose group. The required time for β-hCG levels to decline down to zero was significantly lower in double-dose group. Based on Cut-off points, if initial β-hCG levels are below 3350 single-dose treatment is suggested, while, for the values between 3350 and 3894.5 double-dose treatment, and finally, above 3894.5, other treatments (probably surgery) are suggested.

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