Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(1):397-404
DOI: 10.17826/cumj.1037172

ARAŞTIRMA / RESEARCH

Analysis of multiple-dose methotrexate therapy in tubal ectopic pregnancies

Tubal ektopik gebeliklerde çoklu doz metotreksat tedavisinin analizi

Furkan Çetin[1], Neslihan Bayramoğlu[2], Seyhun Sucu[2], Muhammed Hanifi Bademkıran[2], Ali İrfan Kutlar[2]

[1]Dr. Ersin Arslan Education and Research Hospital, Department of Obstetrics and Gynecology, Gaziantep, Turkey
[2]Gaziantep University School of Medicine, Department of Obstetrics and Gynecology, Gaziantep, Turkey

Cukurova Medical Journal 2022;47(1):397-404

Abstract

Purpose: The study aimed at determining the success rate of sequential multi-dose methotrexate treatment in tubal ectopic pregnancy (EP) patients and the prognostic factors affecting the treatment success.

Materials and Methods: Clinical, laboratory, and demographic data of 63 patients -hospitalized with the diagnosis of EP in our tertiary center between 2017-2020 and administered sequential multiple-dose methotrexate treatment protocol- were analyzed in this retrospective study.

Results: Based on research findings, medical treatment was successful in 45 of the patients. It was found that 16 out of 18-patient, 88.8% of the patients in the failed group with methotrexate treatment, were operated on due to tubal rupture during follow-up. The mean of sonographic free fluid in Douglas of the successful group with methotrexate treatment was found to be 2.40±0.70 mm (Mean±SE), while it was 10.38±3.05 mm (Mean±SE) in the failed group at the first consultation. The initial β-hCG value of the successful group with methotrexate treatment was measured 3668.55±440.55 IU/L (Mean±SE), while it was 4929.72±752.65 IU/L (Mean±SE) in the failed group.

Conclusion: The success rate of the sequential multiple-dose methotrexate protocol was 71.4%. Both the initial serum β-hCG level and the amount of sonographic free fluid in the pouch of Douglas were found high in the failed group with medical treatment at the first consultation.

Keywords: Methotrexate, ectopic pregnancy, tubal rupture

Öz

Amaç: Tubal ektopik gebelik hastalarında ardışık çoklu doz metotreksat tedavisinin başarı oranı ile tedavi başarısına etki eden prognostik faktörlerin belirlenmesi hedeflenmiştir.

Gereç ve Yöntem: Bu retrospektif çalışmada 2017-2020 yılları arasında tersiyer merkezimizde ektopik gebelik tanısı ile hospitalize edilmiş ve ardışık çoklu doz metotreksat tedavi protokolü uygulanmış 63 hastanın klinik, labaratuvar ve demografik verileri analiz edilmiştir.

Bulgular: Hastaların 45'inde medikal tedavi ile başarılı olunmuştur. Metotreksat protokolü ile başarısız olunan hastaların %88.8'inde (n=16) takip sırasında tubal rüptür nedeni ile cerrahi operasyon ihtiyacı gelişmiştir. Medikal tedavi ile başarılı olunan grupta başvuru anında Douglas'ta serbest mayi ortalaması 2.40±0.70 mm (Ort.±SH); başarısız olunan grupta 10.38±3.05 mm (Ort.±SH) bulunmuştur. Medikal tedavi ile başarılı olunan grubun başvuru β-hCG düzeyi 3668.55±440.55 IU/L (Ort.±SH); başarısız olunan grubunki 4929.72±752.65 IU/L (Ort.±SH) bulunmuştur.

Sonuç: Ardışık çoklu doz metotreksat protokolünün başarı oranı %71.4 olarak saptanmıştır. Medikal tedavi ile başarısız olunan grupta başlangıç serum β-hCG düzeyinin yüksek olduğu, yine ilk başvuruda Douglas'ta sonografik olarak ölçülen serbest sıvı miktarının yüksek olduğu tespit edilmiştir.

Anahtar kelimeler: Metotreksat, dış gebelik, tubal rüptür

Yazışma Adresi/Address for Correspondence: Dr. Furkan Çetin, Dr Ersin Arslan Education And Research Hospital, Department of Obstetrics and Gynecology, Gaziantep, Turkey E-mail: furkan.cetin01@gmail.com
Geliş tarihi/Received: 16.12.2021 Kabul tarihi/Accepted: 08.03.2022

INTRODUCTION

Ectopic pregnancy (EP) is the implantation of the fertilized ovum in a location other than the uterine cavity[1,2]. Although the most common settlement is salpinx (95%), it may also show ovarian (3%), cervical, abdominal, intraligamentary, previous cesarean scar, or heterotopic locations[3]. When the EP locations are examined in salpinxes, it was observed that 70% was located in the ampulla, 12% in the isthmus, 11% in the fimbria, and 2-3% in the interstitial or cornual regions[4]. Possible reasons for the increase in the incidence of EP[5] are technological developments facilitating diagnosis, genital tract infections (especially Chlamydia), widespread use of assisted reproductive techniques, systemic diseases disrupting tubal motility, and the use of tubal reanastomosis techniques. Today, despite the developments in diagnosis and treatment, EP that constitutes 1-2% of all pregnancies maintains its importance in terms of maternal mortality and morbidity[6]. In EPs, 90% of maternal mortality occurs due to intraabdominal bleeding[7]. Although the gold standard for diagnosis of EP is laparoscopy, it is often diagnosed by using the serial human chorionic gonadotropin (β-hCG) measurements together with transvaginal sonography[5]. Medical or surgical treatment methods can be used in EP. While determining the treatment method, factors such as the patient's vital signs, β-hCG values, sonographic findings, age, and fertility desire should be taken into consideration. Then, the most appropriate treatment method should be determined[6]. Medical treatment for tubal EP cases is more advantageous than surgical treatment because it is inexpensive, safe and effective with no major side effects[8]. Furthermore, it is less likely to cause tubal damage[5]. Literature survey showed that several authors have emphasized the presence of free fluid of Douglas in EP-diagnosed patients and its crucial effect in planning and evaluation of the treatment[9-11]. In turn, it has been described as a relative contraindication for MTX regimens in the early years of the use of MTX for EP management. Today, it has been accepted that it does not constitute a contraindication for MTX regimens unless there is hemodynamic instability of the EP patient[12].

In this regard, we believe that our study is an important contribution to the literature, showing that the success of MTX treatment decreases in the presence of high amounts of free fluid in Douglas. It is hypothesized that the success rate of multiple-dose MTX treatment decreases in EP patients who have a high amount of free fluid in Douglas at the first sonographic examination. The aim of this study was to determine the success rate of sequential multi-dose methotrexate treatment in tubal EP patients and the prognostic factors affecting the treatment success.

MATERIALS AND METHODS

This retrospective observational study included 63 patients with tubal EP treated by sequential multiple-dose MTX protocol at the Gaziantep University hospital between 2017-2020. The study was approved by the Gaziantep University Ethics Committee (Date: 05 February 2020, Ethics committee number: 2020/03) and informed consent was obtained. This current study had been reviewed by the appropriate ethics committee and had been performed by following the ethical standards described in the current version of the guideline of the Helsinki Declaration.

This study was carried out at the Department of Obstetrics and Gynecology of Sahinbey Research and Practice Hospital which belongs to the Faculty of Medicine of Gaziantep University, Gaziantep, Turkey. Medical and surgical treatments, practices, and measurements were done by the researchers of the Obstetrics and Gynecology Department. Patient information has been stored in the highly secure digital data recording system of the hospital. After reviewing the medical records of the patients who were hospitalized with the diagnosis of EP, among the 157 EP cases, 63 patients treated with multi-dose MTX were selected for the study.

Patient selection criteria and treatment protocol

The diagnosis of tubal EP in all patients in the study was confirmed by positive serum β-hCG (stable or rising serum β-hCG values in separate measurements 48 hours apart) and sonographic markers (empty endometrium, hyperechoic adnexal mass, extrauterine gestational sac with hyperechoic ring, free fluid in Douglas). The study included tubal EP patients who were hemodynamically stable, aged 18-44, had an ectopic mass size smaller than 4 cm, had no contraindication for MTX therapy, and were treated by sequential multi-dose MTX regime. Also, for all of the including patients, the first β-hCG value was <10000 IU/L and had no signs of acute abdomen. While the patients with a history of EP in

their anamnesis treated by follow-up regime (spontaneous regression) were included in the study, those treated medically or surgically were excluded from the study. Also, the patients who had a sonographic fetal cardiac activity in ectopic implantation, were hemodynamically unstable, indicated emergency surgery at the time of diagnosis, and had contraindications for MTX treatment (hepatic or renal failure, thrombocytopenia, anemia, and any suspicion of tubal rupture) were excluded from the study. Therefore, in the period from 2017 to 2020, 63 of 157 EP patients hospitalized in our center were included in the study.

The demographic characteristics, ages, number of gravida, parity, and abortion, body mass indexes (BMI), sonographic signs (ectopic mass size, localization, endometrial thickness, presence and amount of free fluid in Douglas), first and final β-hCG levels, MTX dosages (mg/day) and the number of MTX administrations of the patients were recorded. All patients who were included in the study had pathological results supporting the diagnosis of EP (endometrium sampling results of non-ruptured patients, pathological results of surgical specimens of ruptured patients).

A maximum of 4 doses of MTX, with the highest dose of 80 mg/day, were administered IM every two days (1 mg/kg dose on the 1st, 3rd, 5th, 7th day). Leucovorin (0.1 mg/kg dose on the 2nd, 4th, 6th, 8th days, IM) has been applied to reduce the side effects of MTX. The patients were followed up with consecutive β-hCG measurements (on the 0th, 1st, 3rd, 5th, and 7th day) and hemodynamic parameters.

Grouping criteria of patients and post-treatment management

Sixty-three patients included in the study were divided into two groups. The groups consisted of patients who were successful and failed with the multi-dose MTX treatment (n:45 vs n:18, respectively). Patients with a reduction of more than 15% between two consecutive β-hCG measurements were considered as the successful group. In the successful group, when the β-hCG level decreased sufficiently, treatment was stopped and follow-up was planned until weekly β-hCG follow-ups became negative. The β-hCG level, in which a decrease of more than 15% was observed after the last MTX dose given, was accepted as the final value in patients who were successful with medical treatment. The failed group ıncluded the patients who underwent surgery for EP rupture or whose β-hCG levels did not decrease by more than 15% during follow-up. In the group that failed medical treatment, β-hCG levels that increased or did not decrease more than 15% after administering the last MTX dose were accepted as the final measurement value. Additionally, the β-hCG levels before the surgical operation were accepted as the final measurement value in these patients.

Statistical analysis

Before the study, the approximate sample size was calculated by using Gpower3.1 software. Hence, the minimum number of patients was found 46 in the sample size analysis as per the study by Gnisci et al.[13], with a 95% confidence interval, an effect size of medium, and 80% power. The compatibility of numerical variables to normal distribution was tested with the Shapiro-Wilk test. Normally distributed numerical variables were employed using the Student's t-test in comparing two groups. However, non-normal data sets were compared by the Mann-Whitney-U test. Categorical variables were tested by Chi-square test; furthermore, Fischer Exact test was used when chi-square test conditions were not met statistically. The corrected P-value (P_{adj}) was calculated using the logistic regression method to reduce the effects of age, gravida, parity, abortion, number of previous abdominal operations, EP history, MTX dose amount, and number of administrations on the dependent variable. The R 3.5.1 statistics program (Mathematics and Statistics Institute, Vienna, Austria) package was used in the statistical analysis. In the study, $P \leq 0.05$ was considered statistically significant.

RESULTS

The medical treatment success rate of the patient group was 71.4%. In 88.8% of patients in the failed group, there was an indication for surgery due to ruptured fallopian tubes or intraabdominal abortion during follow-up. The surgical treatment was performed in 11.2% of the failed group because the β-hCG decrease was less than 15% during medical treatment. The clinical parameters and demographic data of the patients are compared based on the groups and results were given in "Table 1". The free fluid in Douglas was positive in 24.4% of the successful group, while it was positive in 55% of the failed group. The amount of free fluid in Douglas of the failed group was significantly higher than the free

fluid of the successful group (P_{adj}=0.009). No significant difference was found between the groups with successful or failed medical treatment in terms of initial β-hCG levels, the number of MTX administrations, and dosages (mg/day) (P_{adj}>0.05). The final β-hCG values were significantly lower in the successful group than in the failed group (P_{adj}=0.05) (Table 2).

Table 1. Comparison of clinical parameters and demographic data in successful and failed groups with sequential multiple-dose MTX treatment

Clinical Parameters and Demographic Data	Medical Treatment Successful Group (n, %) 45 (71.4)	Medical Treatment Failed Group (n, %) 18 (28.6)	P	P_{adj}
Age (Mean±SE)	30.15±0.92	29.72±1.32	0.79	0.62
BMI (Mean±SE)	26.53±0.56	25.94±0.76	0.56	0.72
Gravida (Mean±SE)	3±0.29	3.16±0.39	0.75	0.86
Parite (Mean±SE)	1.26±0.20	1.00±0.25	0.46	0.93
Abortion (Mean±SE)	0.73±0.21	1.11±0.37	0.37	0.98
Abdominal Operation History (n, %)	22 (48.9)	7 (38.9)	0.36	0.32
Ectopic Pregnancy History (n, %)	2 (4.4)	3 (10.7)	0.12	0.18
Endometrium Thickness (mm), (Mean±SE)	10.08±0.61	11.44±1.10	0.25	0.27
Right Ectopic Mass (n, %) Left Ectopic Mass (n, %)	32 (71.1) 13 (28.9)	12 (66.7) 6 (33.3)	0.72	0.43
Ectopic Mass Size (mm), (Mean±SE)	18.11±1.17	20.44±1.94	0.29	0.36
Free Fluid in Douglas (mm), (Mean±SE)	2.40±0.70	10.38±3.05	0.006	0.009

(n, %): Number of Patients in the Group and Percentage, Mean±SE: Mean ± Standard Error; SE: Standard Error of the Mean ($\frac{S_{\bar{x}}}{\sqrt{n}}$), P_{adj}: Adjusted P-Value; BMI: Body Mass Index

Table 2. Comparison of MTX dosages, number of doses and β-hcg levels in successful and failed groups with sequential multiple-dose MTX treatment

Variables	Medical Treatment Successful Group (n, %) 45 (71.4)	Medical Treatment Failed Group (n, %) 18 (28.6)	P	P_{adj}
Number of MTX Doses			0.80	0.61
1	6 (13.3)	3 (16.7)		
2	15 (33.3)	5 (27.8)		
3	8 (17.8)	2 (11.1)		
4	16 (35.6)	8 (44.4)		
MTX Dosage			0.75	0.51
60 mg	20 (44.4)	9 (50)		
65 mg	1 (2.2)	5 (27.8)		
70 mg	16 (35.6)	3 (16.7)		
75 mg	2 (4.4)	1 (5.6)		
80 mg	6 (13.3)	0		
First β-hCG Value (IU/L) (Mean±SE)	3668.55±440.55	4929.72±752.65	0.14	0.20
Final β-hCG Value (IU/L) (Mean±SE)	2022.75±259.35	3115.11±551.61	0.05	0.05

(n, %): Number of Patients in the Group and Percentage, Mean±SE: Mean ± Standard Error; SE: Standard Error of the Mean ($\frac{S_{\bar{x}}}{\sqrt{n}}$), P_{adj}: Adjusted P-Value; MTX: Methotrexate, β-hCG: Human Chorionic Gonadotropin

Research results indicated that 14.2% of 63 patients were treated by single-dose, 31.7% two-dose, 15.8% three-dose, and 38.3% four-dose MTX. In order to evaluate the relationship between the number of MTX administrations and the change of β-hCG, the single-dose group versus the four-dose group was compared. While the first β-hCG value of the single-dose group was 1716.22±730.34 IU/L (Mean±SE), the final β-hCG value was found to be 754.00±364.55 IU/L (Mean±SE). The first β-hCG value of the four-dose group was 4969.50±651.68 IU/L (Mean±SE), while the final β-hCG value was found to be 3075.75±464.07 IU/L (Mean±SE). The decrease in the β-hCG value was 56.06% in the single-dose group and 38.10% in the four-dose group. Statistically, the decrease in the β-hCG value was found to be significantly higher in the single-dose MTX group compared to the four-dose group (P=0.05). However, there was no statistically significant relationship between the MTX dosages (mg/day) and the changes in β-hCG levels (P=0.91).

DISCUSSION

In the study, the success rate was found to be 71.4% in patients who received sequential multiple-dose methotrexate treatment due to EP. It was concluded that the initial β-hCG level and the amount of free fluid in Douglas may be predictive factors that play an important role in treatment failure.

It is thought that the risk of EP will increase as the exposure to risk factors of EP increases with advancing age[14]. The mean age of the EP patients included in the study was found to be 30.03±0.77 (Mean±SE) in line with the general literature. Goksedef et al.[15] reported that the parallelism between increasing age and the incidence of EP was not observed for treatment failure. On the other hand, Roya et al.[16] reported that there has been a correlation between advancing age and treatment failure. In this current study, the mean age of the patients who were successful with the sequential multiple-dose MTX protocol was 30.15±0.92 (Mean±SE), while the mean age of the patients who failed the treatment was 29.72±1.32 (Mean±SE). Furthermore, it was found that the age factor did not make any statistically significant contribution to the success of the treatment (P_{adj}>0.05).

The study conducted by Lawlor et al.[17] pointed out that previous EP history increased the risk of EP approximately 7-9 times, and approximately 10% of EP patients had a history of EP. Similarly, our study indicated that only 7.9% of the patients had a history of EP. This rate was found compatible with the literature, but relatively low regarding the percentage. Considering the patients with EP history, there was no statistically significant difference between the two groups with or without treatment success (P_{adj}>0.05). Contrary to the results of our study, it has been reported in the literature that the history of EP reduces the success of medical treatment[18]. The apparent difference between the literature and the results of our study might be due to the fact that all cases with previous EP history were not included in the current study.

Bowman et al.[19] reported that previous cesarean sections do not cause an increase in the risk of EP. However, it is noteworthy that some studies have reported contrasting cases. For example, the previous non-gynecological abdominal surgeries cause peritubal scarring due to interventions performed close to the peritubal region. In turn, this practice increases the risk of EP development at a low level[20]. Patients who had undergone ovarian or salpinx surgery before were not included in this study. It is important to highlight that 53.9% of the patients had not undergone abdominal surgery before. In the current study, in addition to the non-gynecological abdominal operation history, it was determined that the number of gravida, parity, and abortus of the patients did not differ significantly in the success of medical treatment (P_{adj}>0.05).

When the previous studies are examined, it is understood that the success rate of MTX treatment in ectopic pregnancies varies between 65% and 94%[21-23]. In this study, the success rate of sequential multi-dose MTX therapy was found to be 71.4% which is rather consistent with the literature. In a systemic review by Yang et al.[23], the pre-treatment β-hCG level was reported to be the most valuable prognostic marker which is used to predict treatment success. In addition, it has been demonstrated that the success rate of single-dose MTX decreased and the need for multiple doses of MTX or surgery increased in patients whose initial serum β-hCG level was above 5000 IU/L. Literature survey[24-27] shows that the medical treatment success rate decreases in EP patients with a β-hCG value above 5000 IU/L at the time of diagnosis. In addition, it has been reported that the success of medical treatment could not be appraised acknowledgeably because the

majority of these patients were treated surgically, resulting in the lack of data. In our study, the initial β-hCG value of the successful group with medical treatment was 3668.55±440.55 IU/L (Mean±SE) while it was 4929.72±752.65 IU/L (Mean±SE) for the failed group. In addition to that, the first β-hCG level of the group that failed medical treatment was compatible with the cut-off value (β-hCG: 5000 IU/L) of medical treatment failure in the literature. Although the β-hCG value at the time of admission of the successful group with medical treatment was lower than that of the failed group with medical treatment, comparison tests of our study indicated that there was no statistically significant difference (P_{adj}>0.05) between the initial β-hCG levels of the two groups. While the final β-hCG value was 2022.75±259.35 IU/L (Mean±SE) in the group whose medical treatment was successful, it was 3115.11±551.61 IU/L (Mean±SE) in the failed group. The final β-hCG value of the medical treatment successful group was found to be significantly lower than the failed group's final β-hCG value (P_{adj}=0.05). In line with the results of previous studies, given that MTX treatment efficiency increases at the low initial β-hCG values, the fact that the final β-hCG value in the successful group was found to be significantly lower than the failed group might be attributed to the low initial β-hCG values of the successful group. On the whole, research findings were found to be compatible with the literature.

In the study, no significant difference was observed in terms of the number and amount of MTX doses (mg/day) in the success or failure of the treatment (P_{adj}>0.05). In order to make comprehensive evaluations of the changes in the β-hCG levels by the number of MTX doses, the patients who received the minimum and the maximum number of doses were examined separately. A 56.06% decrease was observed in the β-hCG level of the single-dose group and a 38.10% decrease in the four-dose group. The β-hCG reduction was significantly higher in the single-dose MTX group (P=0.05). In accordance with the information in the literature[21,23], the EP patients with low initial β-hCG levels are usually treated with a high treatment efficacy without the need for extra doses of MTX, which may be the reason for this result found in our study. Mavrelos et al.[28] reported that the development of tubal abortion or rupture in EP cases reduced the level of β-hCG. Based on the results of our study, there was a 44.87% decrease in β-hCG levels of the successful group and 36.80% in the failed group. In the failed group, 16 out of 18-patient (88.8% of the patients) were found to be operated on due to tubal rupture or tubal abortion during follow-up. The remaining 11.2% of these patients underwent surgery because the β-hCG value did not decrease during MTX treatment. Similar to the successful group, the observation of the β-hCG decrease in the failed group at these rates was associated with a decrease in the β-hCG as a result of the rupture of the EP material from the implantation site.

In a study on EP localization conducted by Sudha et al.[29], it was reported that right tubal ectopic pregnancies were observed more frequently. On the other hand, there was no significant relationship between EP localization and treatment success or failure (P_{adj}>0.05). Sindiani et al.[21] reported that the size of the ectopic mass did not affect the medical treatment results. On the contrary, Celik et al.[30] reported that the increased size of the ectopic mass decreased the success of the treatment and that the endometrial thickness did not affect the treatment results. In this study, the size of the ectopic mass and the measure of endometrial thickness did not have any significant impact on the treatment success (P_{adj}>0.05). Some studies have reported that the presence of free fluid in Douglas might be associated with EP. However, this is not a reliable indicator of EP rupture[31,32]. Var et al.[33] concluded that the presence of intraperitoneal fluid did not affect the success of the treatment. Sindiani et al.[21] reported that free fluid in the pelvis was not an important risk factor for medical treatment failure. Similarly, Celik et al.[30] reported that the presence of free fluid in Douglas did not increase medical treatment failure. On the contrary, there have been recent studies[34,35] reporting that the sonographic free fluid in Douglas could be considered as a relative contraindication for MTX treatment. In our study, 24.4% and 55.5% of patients in the successful and failed groups, respectively, had sonographic free fluid in Douglas at the first consultation. While the amount of free fluid in Douglas was 2.40±0.70 mm (Mean±SE) in the successful group, it was approximately 4 times higher, 10.38±3.05 mm (Mean±SE), in the failed group. There was a statistically significant difference (P_{adj}=0.009) in the amount of free fluid in Douglas between successful and failed groups. Sivalingam et al.[35] reported parallelism between serum β-hCG levels and the amount of free fluid in Douglas in medical treatment failure in ectopic pregnancies. Similarly, in this current study, the initial serum β-hCG level and the amount of free fluid in the

pouch of Douglas were higher in the failed group as compared with the values of the successful group. These findings imply that the patients with a large amount of free fluid in Douglas might be exposed to medical treatment failure.

This research was conducted as part of a dissertation for the degree of Doctor of Philosophy in Obstetrics and Gynecology at Gaziantep University, Faculty of Medicine, Turkey. The current study consists of data from a single-center, and the number of cases is relatively low due to the patient selection criteria of the study. These can be listed among the reasons that negatively affect the reliability of the study. Since there was no need for multiple doses of MTX because of low initial β-hCG values, it is understood that the majority of studies examining the relationship between MTX treatment and the free fluid in Douglas were conducted with the single-dose MTX. In our study, most of the patients were treated with multiple doses of MTX. In addition, the initial β-hCG levels of the patients were found high. We believe that the strength of this study is the fact that it is performed on the patients who were administered multiple doses of MTX. Another strength of the study is that the Department of Obstetrics and Gynecology at Gaziantep University is an experienced center in EP treatment. Considering that the results available in the literature are also controversial, the hypothesis of the correlation between the amount of free fluid in Douglas and the failure of the sequential multi-dose MTX protocol requires further and detailed research. Consequently, we think that it needs confirmation with randomized studies with larger cases. It is also important to note that 88.8% of patients, who failed medical treatment, was in need of surgery due to tubal rupture during follow-up. Hospitalization and close observation of patients who will receive sequential multiple-dose MTX therapy is a more rational and appropriate approach.

In conclusion, the success rate of the sequential multi-dose MTX treatment of 63 tubal EP patients was 71.4% in this study. This success rate supports the importance of sequential multi-dose MTX protocol of patients with selected tubal EP. In the treatment of EP, a high β-hCG value at the time of diagnosis is among the main prognostic factors affecting the success rate of the treatment. In parallel with the many studies in which low success rates of medical treatment and high initial serum β-hCG values were observed, our present study supported that the initial β-hCG value was high for the patients with medical treatment failure. Moreover, the results indicated that the presence and amount of free fluid in Douglas may reduce the success rates of medical treatment.

Yazar Katkıları: Çalışma konsepti/Tasarımı: FÇ, NBT, SS, MHB, AİK; Veri toplama: AİK, NBT; Veri analizi ve yorumlama: AİK, SS; Yazı taslağı: FÇ; İçeriğin eleştirel incelenmesi: NBT, SS; Son onay ve sorumluluk: FÇ, NBT, SS, MHB, AİK; Teknik ve malzeme desteği: MHB; Süpervizyon: FÇ, NBT; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Gaziantep Üniversites i Klinit Araştırmalar Etik Kurulundan 05.02.2020 tarih ve 2020/03 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması olmadığını beyan etmemişlerdir.
Finansal Destek: Yazarlar bu çalışmanın finansal destek almadığını beyan etmişlerdir.

Author Contributions: Concept/Design : : FÇ, NBT, SS, MHB, AİK; Data acquisition: AİK, NBT; Data analysis and interpretation: AİK, SS; Drafting manuscript: FÇ; Critical revision of manuscript: NBT, SS; Final approval and accountability: FÇ, NBT, SS, MHB, AİK; Technical or material support: MHB; Supervision: FÇ, NBT; Securing funding (if available): n/a.
Ethical Approval: For this study, ethical approval was obtained from the Clinical Research Ethics Committee of Gaziantep University by its decision dated 05.02.2020 and numbered 2020/03.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors declared no conflict of interest.
Financial Disclosure: The authors declared that this study received no financial support

REFERENCES

1. Eriç HJ, Görkemli H, Özkaya EB, Işıkalan MM, Akıncı D, Kılıç F. Ektopik gebelik ve düşük tehdidi tanısında hemogram parametrelerinin değerlendirilmesi. Cukurova Med J. 2021;46:63-9.
2. Cunningham F, Leveno K, Bloom S, Spong CY, Dashe J.. Ectopic Pregnancy. In Williams Obstetrics, 24th ed., New York, McGraw-Hill, 2014.
3. Desroque D, Capmas P, Legendre G, Bouyer J, Fernandez H. Fertility after ectopic pregnancy. J Gynecol Obstet Biol Reprod (Paris). 2010;39:395-400.
4. Spandana J, Tejaswini D, Bai GS. Clinical course of ectopic pregnancy: a tertiary centre experience. Int J Reprod Contracept Obstet Gynecol. 2017;6:2990-7.
5. Baselice J, Yates M. Ectopic pregnancy. In The Johns Hopkins Manual of Gynecology and Obstetrics (Eds Hurt KJ, Guile MW, Bienstock JL, Fox HE, Wallach EE):340-9. New York, Lippincott Williams & Wilkins, 2012.
6. van Mello NM, Mol F, Ankum WM, Mol BW, van der Veen F, Hajenius PJ. Ectopic pregnancy: how the diagnostic and therapeutic management has changed. Fertil Steril. 2012;98:1066-73.
7. Creanga AA, Shapiro-Mendoza CK, Bish CL, Zane S, Berg CJ, Callaghan WM. Trends in ectopic pregnancy mortality in the United States: 1980-2007. Obstet Gynecol. 2011;117:837-43.

8. Dhar H, Hamdi I, Rathi B. Methotrexate treatment of ectopic pregnancy: experience at nizwa hospital with literature review. Oman Med J. 2011;26:94-8.
9. Thacker J, Reardon R. Sonographic free fluid in patients with ectopic pregnancy. Ann Emerg Med. 2004;44:85.
10. Sargin MA, Yassa M, Taymur BD, Çelik A, Aydin S, Orhan E et al. A clinical experience of ectopic pregnancies with initial free intraperitoneal fluid. J Clin Diagn Res. 2016;10:22-6.
11. Phatak S, Shrivastav D, Marfani G, Daga S, Madurwar K, Samad S. Transvaginal sonography and elastography evaluation of ectopic pregnancy. J Datta Meghe Instit Med Sci Univ. 2019;14:86-9.
12. Practice Committee of American Society for Reproductive Medicine. Medical treatment of ectopic pregnancy: a committee opinion. Fertil Steril. 2013;100:638-44.
13. Gnisci A, Stefani L, Bottin P, Ohannessian A, Gamerre M, Agostini A. Predictive value of hemoperitoneum for outcome of methotrexate treatment in ectopic pregnancy: an observational comparative study. Ultrasound Obstet Gynecol. 2014;43:698-701.
14. Moini A, Hosseini R, Jahangiri N, Shiva M, Akhoond MR. Risk factors for ectopic pregnancy: a case–control study. J Res Med Sci. 2014;19:844-9.
15. Goksedef BP, Kef S, Akca A, Bayik RNE, Cetin A. Risk factors for rupture in tubal ectopic pregnancy: definition of the clinical findings. Eur J Obstet Gynecol Reprod Biol. 2011;154:96-9.
16. Roya FD, Maryam A, Nastaran FP, Ali AA. Predictive value of maternal serum beta-hCG concentration in the ruptured tubal ectopic pregnancy. Iran J Reprod Med. 2015;13:101-6.
17. Lawlor DA, Mortensen L, Andersen AM. Mechanisms underlying the associations of maternal age with adverse perinatal outcomes: a sibling study of 264 695 Danish women and their firstborn offspring. Int J Epidemiol. 2011;40:1205-14.
18. Bonin L, Pedreiro C, Moret S, Chene G, Gaucherand P, Lamblin G. Predictive factors for the methotrexate treatment outcome in ectopic pregnancy: a comparative study of 400 cases. Eur J Obstet Gynecol Reprod Biol. 2017;208:23-30.
19. Bowman ZS, Smith KR, Silver RM. Cesarean delivery and risk for subsequent ectopic pregnancy. Am J Perinatol. 2015;32:815-20.
20. Olooto WE, Amballi AA, Banjo TA. A review of female infertility; important etiological factors and management. J Microbiol Biotech Res. 2012;2:379-85.
21. Sindiani AM, Alshdaifat E, Obeidat B, Obeidat R, Rawashdeh H, Yaseen H. The use of single dose methotrexate in the management of ectopic pregnancy and pregnancy of unknown location: 10 years' experience in a tertiary center. Int J Womens Health. 2020;12:1233-39.
22. Cohen A, Zakar L, Gil Y, Amer-Alshiek J, Bibi G, Almog B et al. Methotrexate success rates in progressing ectopic pregnancies: a reappraisal. Am J Obstet Gynecol. 2014;211:128.
23. Yang C, Cai J, Geng Y, Gao Y. Multiple-dose and double-dose versus single-dose administration of methotrexate for the treatment of ectopic pregnancy: a systematic review and meta-analysis. Reprod Biomed Online. 2017;34:383-91.
24. Skubisz MM, Tong S. The evolution of methotrexate as a treatment for ectopic pregnancy and gestational trophoblastic neoplasia: a review. ISRN Obstet Gynecol. 2012;2012:637094.
25. Bachman EA, Barnhart K. Medical management of ectopic pregnancy: a comparison of regimens. Clin Obstet Gynecol. 2012;55:440-7.
26. Cecchino GN, Araujo Júnior E, Elito Júnior J. Methotrexate for ectopic pregnancy: when and how. Arch Gynecol Obstet. 2014;290:417-23.
27. Hamed HO, Ahmed SR, Alghasham AA. Comparison of double- and single-dose methotrexate protocols for treatment of ectopic pregnancy. International Journal of Gynecology & Obstetrics. 2012;116:67-71.
28. Mavrelos D, Nicks H, Jamil A, Hoo W, Jauniaux E, Jurkovic D. Efficacy and safety of a clinical protocol for expectant management of selected women diagnosed with a tubal ectopic pregnancy. Ultrasound Obstet Gynecol. 2013;42:102-7.
29. Sudha VS, Thangaraj DR. A retrospective study on ectopic pregnancy: a two year study. Int J Reprod Contracept Obstet Gynecol. 2016;5:4365-9.
30. Celik E, Türkçüoğlu I, Karaer A, Kırıcı P, Eraslan S. Assessment of early decline in the percentage of β-hCG values between days 0 and 4 after methotrexate therapy in ectopic pregnancy for the prediction of treatment success. J Turk Ger Gynecol Assoc. 2013;14:125-9.
31. Orazulike NC, Konje JC. Diagnosis and management of ectopic pregnancy. Womens Health (Lond). 2013;9:373-85.
32. Lee R, Dupuis C, Chen B, Smith A, Kim YH. Diagnosing ectopic pregnancy in the emergency setting. Ultrasonography. 2018;37:78-87.
33. Var A, Özyurt R, Şık BA, Kumbasar S, Sever E, Deveci M et al. Retrospective analysis of factors that affect the success of single-dose methotrexate treatment in ectopic pregnancy. Turk J Obstet Gynecol. 2015;12:215-9.
34. Lermann J, Segl P, Jud SM, Beckmann MW, Oppelt P, Thiel FC et al. A. Low-dose methotrexate treatment in ectopic pregnancy: a retrospective analysis of 164 ectopic pregnancies treated between 2000 and 2008. Arch Gynecol Obstet. 2014;289:329-35.
35. Sivalingam VN, Duncan WC, Kirk E, Shephard LA, Horne AW. Diagnosis and management of ectopic pregnancy. J Fam Plann Reprod Health Care. 2011;37:231-40.