Northwestern Med J.2021;1(2):53-62
doi: 10.5222/NWMJ.2021.26349

Original Article

Decrease of Serum hCG on Day 4 of Single-dose Methotrexate Regimen is Valuable to Predict Treatment Failure in Patients with Intact Tubal Ectopic Pregnancy

Tek Doz Metotreksat Rejiminin 4. Gününde Serum hCG'sinin Azalması, İntakt Tubal Ektopik Gebeliği Olan Hastalarda Tedavi Başarısızlığını Öngörmede Değerlidir

Fırat Ekmez, Murat Ekmez

Cite as: Ekmez F, Ekmez M. Decrease of serum hCG on day 4 of single-dose methotrexate regimen is valuable to predict treatment failure in patients with intact tubal ectopic pregnancy. Northwestern Med J. 2021;1(2):53-62.

ABSTRACT

Objective: We aimed to determine the predictivity of decrease in serum hCG value on day 4 of single-dose methotrexate (MTX) regimen for the need for surgery in patients with intact tubal ectopic pregnancy (TEP).
Method: We evaluated 41 patients diagnosed with intact TEP and treated with single-dose intramuscular injection of MTX treatment according to body surface area (50 mg/m^2). Serum hCG values on days 0, 4, and 7 were retrieved to determine patterns of hCG change. Clinical data of patients with treatment success or failure were analyzed.
Results: Of 41 patients, 31 was successfully treated with MTX administration, and in the rest (n=10), surgery was required because of intraabdominal hemorrhage. On days 0, 4, and 7, the median serum hCG values in patients with treatment failure were significantly higher compared to treatment success ($p<0.05$). In the patients with treatment failure, the median hCG values on day 7 was significantly lower than that on day 4 ($p<0.05$).
Conclusion: During the follow-up of patients with intact TEP who administered single-dose MTX regimen, decrease of serum HCG value on day 4, in addition to low HCG value and endometrial thickness on day 0, is valuable to predict treatment failure.

Keywords: tubal ectopic pregnancy, ectopic pregnancy, hCG, methotrexate, treatment failure, tubal surgery

ÖZ

Amaç: Bu retrospektif çalışmada, intakt tubal ektopik gebelikte (TEG) tek doz metotreksat (MTX) tedavisinin 4. günündeki serum hCG değerinin tedavi başarısızlığındaki öngörülebilirliğini belirlemeyi amaçladık.
Yöntem: Bu retrospektif çalışmada, intakt TEG tanısı alan ve vücut yüzey alanına (50 mg/m^2)göre tek doz intramüsküler MTX tedavisi ile tedavi edilen 41 hasta değerlendirildi. hCG değişim modellerini belirlemek için 0, 4 ve 7. günlerdeki serum hCG değerleri alındı. Hastaların tıbbi kayıtlarından tubal ektopik gebelik ile ilgili klinik ve laboratuvar verileri toplandı. Tedavi başarısı veya başarısızlığı olan hastaların klinik verileri analiz edildi.
Bulgular: 41 hastanın 31'i MTX uygulaması ile başarılı bir şekilde tedavi edildi ve geri kalanında (n=10) batın içi kanama nedeniyle operasyon gerekti. Tedavisi başarılı olan hastalarda gebelik dışı serum hCG değerlerine ulaşma süresi 22 (15-26) gündü. Tedavisi başarısız olan hastaların endometrial kalınlıkları tedavisi başarılı olan hastalara kıyasla anlamlı olarak daha yüksekti. 0, 4 ve 7. günlerde, tedavisi başarısız olan hastalarda median serum hCG değerleri tedavisi başarılı olan hastalara göre anlamlı derecede yüksekti ($p<0.05$). Tedavisi başarılı olan hastalarda, 7. gündeki median serum hCG değerleri 4. ve 0. günlere göre anlamlı derecede düşüktü ($p<0.05$). Tedavisi başarısız olan hastalarda 7. gündeki median hCG değerleri 4. güne göre anlamlı derecede düşüktü ($p<0.05$).
Sonuç: Tek doz MTX rejimi uygulanan intakt TEG hastaların takibinde 4. günde serum HCG değeri düşüşü, 0. günde düşük serum hCG değeri ve endometrial kalınlığa ek olarak tedavi başarısızlığını öngörmede değerlidir.

Anahtar kelimeler: tubal ektopik gebelik, ektopik gebelik, hCG, metotreksat, tedavi başarısızlığı, tubal cerrahi

Received: 29.07.2021
Accepted: 31.07.2021
Publication date: 03.09.2021

Corresponding Author:
Fırat Ekmez
Private Clinic, Yesiltepe St., Silopi, Sirnak
firatekmez@hotmail.com
ORCID: 0000-0001-8076-1632

M. Ekmez 0000-0001-5045-3831
Health Science University, Haseki Trainig and Research Hospital, Department of Obstetrics and Gynecology, İstanbul

© Copyright Northwestern Medical Journal of Medicine. This journal published by Logos Medical Publishing.
Licenced by Creative Commons Attribution 4.0 International (CC BY)

INTRODUCTION

Ectopic pregnancies which occur outside of the uterine cavity account for 2% of all pregnancies, and in approximately 90% of cases, the fallopian tube is their location (1,2,3). Prior tubal ectopic pregnancy (TEP), history of pelvic inflammatory illness, current intrauterine device use, any tubal surgery, in utero diethylstilbestrol exposure and smoking are all risk factors. However, approximately half of the TEP patients have none of these risk factors (3,4,5). Advancements in ultrasound imaging and development of protocols to screen women with early pregnancy have led to earlier detection of TEPs. The options for the treatment of TEP patients may be medical, surgical, and surveillance. As more women with intact TEP appear as clinically stable without concern for tubal rupture, preferred treatment modalities have expanded beyond surgical management to medical management (3,5). Medical therapy success rates are approaching 90% and following tubal patency rates on hysterosalpingograms are above 80% (6).

Beyond intramuscular methotrexate (MTX), which has cytotoxic effects on actively dividing cells like trophoblasts, there are no recognized alternative medical treatment methods for ectopic pregnancy. MTX, a folate antagonist, and its active metabolites, MTX polyglutamates, bind to the catalytic site of dihydrofolate reductase and they can interrupt the synthesis of purine nucleotides and block DNA synthesis and tissue repair and cell proliferation (1,7). For MTX administration, intramuscular injection is most common route. There are three types of MTX regimens: single-dose, two-dose, and multi-dose. Because it does not require the administration of leucovorin and requires fewer clinic visits, the single-dose regimen is more convenient than the multi-dose and two-dose regimens. The single-dose regimen is the simplest of the three; nevertheless, in up to a quarter of patients, an additional dose may be required to guarantee resolution. hCG levels monitoring and ultrasound scans are used to identify TEP patients before they develop clinical signs. Despite improved awareness of TEPs and advancements in their care, 5–30% of patients are at risk of secondary surgery or supplementary dosage due to failure of the first MTX administration, which continues to be a major cause of pregnancy-related mortality and morbidity (1,7,8).

MTX regimens are monitored with serum hCG measurements until the decrease of hCG to non-pregnant levels. Serum hCG levels may initially increase but then progressively diminish, highlighted by at least 15% decrease by 4 to 7 days after start of treatment. This is used as main criterion to accept the medical therapy as successful (1,4,5,9,10,11).

Despite continuing research activities, in the management of TEPs in conventional clinical settings, the clinical success of medical treatment of TEPs is not reached the expected level and the requirement of surgical interventions is still an important need in patients with the complications of TEP such as tubal rupture, abortion, and hemorrhage. One of the reasons leading to the suboptimal treatment of TEP with single-dose MTX regimen may be the use of conventional laboratory workup, including mainly the measurement times of serum hCG and how these hCG values can be interpreted. These diagnostic and clinical dilemmas can lead the gynecologists to the preference of inpatient management longer than required in patients with intact TEPs, decreasing cost-effectiveness of management regimen.

Additionally, during the single-dose MTX regimen, there can be a need for administration of second-dose MTX in some patients with suboptimal serum hCG decrease. During waiting for decision-making with serum hCG level on day 7, there is somewhat an increase in the number of patients with the development of tubal hemorrhage and need for surgical intervention. Considering the pertinent data in the literature, there are patients with intact TEP as well as a wide range of serum hCG values with different basal levels and trends during their follow up, depending on loss of viability and the beginning of tubal abortion process and these variations may be important factors affecting the success of

single-dose MTX regimen. In routine clinical practice, the change of serum hCG value measured on day 7 compared to day 4 is used to change the management strategy in patients with intact TEP. Considering the importance of early decision-making and reducing the need for surgical interventions, we think that evaluation of serum hCG values measured on day 4 can also be helpful to detect patients in whom the single-dose MTX regimen is unsuccessful and there is a need for administration of second-dose MTX to prevent surgical interventions. In this retrospective study, we aimed to determine the predictivity of decrease in serum hCG value on day 4 of single-dose MTX regimen for the need for surgery in patients with intact TEP.

MATERIALS AND METHODS

In this retrospective study, we retrieved the hospital records of patients with intact TEP who underwent single-dose intramuscular MTX treatment from September 2008 to September 2012 in the Süleymaniye Gynecology and Pediatrics Training and Research Hospital. The EP was evaluated and diagnosed using the findings of standard clinical examinations, transvaginal ultrasound, and serial serum hCG measurements. The following were the inclusion criteria for MTX treatment: (1) hemodynamic stability; (2) presence of extra-uterine gestational sac or absence of an intrauterine pregnancy despite serum hCG value of ≥1500 mIU/mL and a continuous increase in the hCG values; (3) absence of free fluid in the pelvis with signs of acute abdomen or hemoperitoneum as assessed by transvaginal ultrasound; (4) absence of fetal heartbeat in the extra-uterine sac; (5) serum hCG level of <10,000 mIU/mL; (6) size of the tubal mass <3.5 cm; (7) absence of hepatic dysfunction (high levels of liver function tests) or renal insufficiency (creatinine level of >1.5 mg/dL); and (8) absence of a known allergy to MTX. Exclusion criteria were the following: allergy to MTX and missing clinical data.

In our clinic, according to the protocol of 9, single-dose MTX regimen is routinely aused in the treatment of ectopic pregnancy at a dose of 50 mg/m2 of body surface area. This protocol is used in many centers throughout the world. On day 7, a complete blood count, liver, and renal function tests were performed. All patients were urged not to engage in sexual intercourse. The patients were instructed to notify clinical professionals if their abdomen pain increased significantly. All women were also told to consume more water and stay out of the sunshine. They were also recommended to avoid alcohol, nonsteroidal anti-inflammatory medications, and aspirin, and informed about the common side effects of MTX. The treatment success of single dose MTX regimen was defined conventionally as a decrease in serum hCG value ≥15% between 4 and 7 days and no surgery or no second MTX dose required until the β-hCG value returned to non-pregnant levels with the resolution of tubal mass.

Patterns of serum hCG values from days 0 to 4 and from days 4 to 7 were analyzed. Clinical data related to TEP were recorded: age, parity, gestational age, ultrasonographic diameter of tubal mass, location of gestational sac, endometrial thickness, days required to reach to normal serum hCG values, histories of ectopic pregnancy and tubal surgery, smoking, and intrauterine device usage. Noteworthy, no severe adverse events related to MTX occurred during the study period.

Statistical analysis

SPSS for Windows version 22.0 was used for all statistical analyses (IBM Corp., Armonk, NY USA). The Kolmogorov-Smirnov test was used to determine whether the data distribution was normal. Continuous variables are represented by a mean, standard deviation, and median (25th–75th percentile), while categorical variables are represented by a number and a percentage. Comparisons of continuous variables between groups were performed using the Mann-Whitney test or repeated measures ANOVA test with post hoc Dunn's test. The chi-square test was used to make comparisons between groups of categorical variables. Statistical significance was defined as a two-sided p value of less than 0.05.

Table 1. Selected clinical data of patients in patients with intact tubal ectopic pregnancy whose medical therapy was successful and who required tubal surgery because of abdominal hemorrhage during the administration of single-dose methotrexate regimen.

	Successful treatment (n=31)	Surgery required (n=10)	p
Age (y)	33.16±5.61 34 (30-37)	31.20±4.02 30.5 (28.75-33.25)	0.170
Parity (n)	1.26±1.29 1 (0-2)	0.6±0.84 0 (0-1.25)	0.151
Gestational age (d)	42.68±8.05 43 (35-49)	42.5±6.04 43.5 (37.75-45.5)	0.927
Ultrasonographic diameter of ectopic pregnancy mass	21.45±5.37 21 (19-25)	21.3±4.32 20.5 (18.75-25.25)	0.915
Location of gestational sac Left Right	 14 (45.2%) 17 (54.8%)	 5 (50%) 5 (50%)	 p=0.790
Endometrial thickness (mm)	6.98±2.42 6 (5-8)	9.6±3.81 9 (6.75-12.75)	0.037
Days required to reach to normal serum hCG values	22.39±8.5 22 (15-26)		
History of ectopic pregnancy No Yes	 27 (87.1%) 4 (12.9%)	 9 (90%) 1 (10%)	 p=0.807
History of tubal surgery No Yes	 29 (93.5%) 2 (6.5%)	 9 (90%) 1 (10%)	 p=0.708
Smoking No Yes	 18 (58.1%) 13 (41.9%)	 3 (30%) 7 (70%)	 p=0.123
IUD usage No Yes	 29 (93.5%) 2 (6.6%)	 7 (77.8%) 2 (22.2%)	 p=0.165

Data were presented as mean with standard deviation, median with interquartile range, or number (%) as appropriate and analyzed with Mann-Whitney and chi-square tests as appropriate. IUD. intrauterine device.

RESULTS

Data analyses were performed with 41 patients with intact TEP who were administered intramuscular MTX 50 mg/m^2. These patients were divided into two groups according to the need for surgical treatment during the follow-up period of MTX treatment. Of these 41 patients, 31 was successfully treated with MTX administration, and in all the remaining patients (n=10), surgical treatment of TEP was required because of intraabdominal hemorrhage.

Table 1 presents the selected clinical data of the study population. The median age, parity, and diameter of ectopic pregnancy mass; and the rates of histories of ectopic pregnancy and tubal surgery, location of gestational sac, smoking, and IUD usage were found similar ($p>0.05$). In the patients with treatment failure, the median endometrial thickness was significantly higher compared to treatment success, in accordance with the high serum hCG values in patients with treatment failure ($p<0.05$). The time required to reach to non-pregnant serum hCG values was 22 (15-26) days in the patients treated successfully with MTX administration.

Figure 1 shows the median serum hCG values obtained as baseline at admission before MTX administration and 4 and 7 days later after MTX administration in patients with intact TEP whose medical therapy was successful and who required tubal surgery because of abdominal hemorrhage during MTX treatment. On days 0, 4, and 7, the median serum hCG values in patients undergone tubal surgery were significantly higher than those in patients treated successfully with MTX administration [3011 (1015.75-3675) vs. 750 (352-1749) on day 0; 2895 (1681-4170) vs. 897 (335-1800) on day 4; 1835 (1023-2587) vs. 493 (250-1350) on day 7; all p values were less than 0.05)]. In the patients treated successfully with MTX administration, the median serum hCG values on day 7 was significantly lower than those on days 4 and 0 [493 (250-1350) vs. 897 (335-1800) and 750 (352-1749), respectively, all p values were less than 0.05]. In the patients undergone tubal surgery because of abdominal hemorrhage during MTX treatment, the median hCG values on day 7 was significantly lower than that on day 4 [1835 (1023-2587) vs. 2895 (1681-4170); $p<0.05$].

Overall, when the serum hCG data were evaluated in patients treated successfully with MTX administration, firstly, there was a non-significant increase on day 4 ($p>0.05$) and later a significant decrease on day 7 ($p<0.05$). Contrary to this result, in patients undergone tubal surgery because of abdominal hemorrhage during MTX treatment, there was a progressive decrease in the median serum hCG values from day 0 to day 7 ($p<0.05$). In patients undergone tubal surgery because of abdominal hemorrhage during MTX treatment, 25% interquartile of serum hCG was higher than 1000 mIU/mL; however, majority of patients treated successfully with MTX administration had lower hCG values than 1000 mIU/mL.

Table 2 shows the change in the serum hCG values on day 4 versus day 0 in the study groups. The rate of decrease on day 4 in the patients with intact TEP whose medical therapy was successful with MTX administration compared to in patients who required tubal surgery because of abdominal hemorrhage during MTX treatment ($p<0.05$).

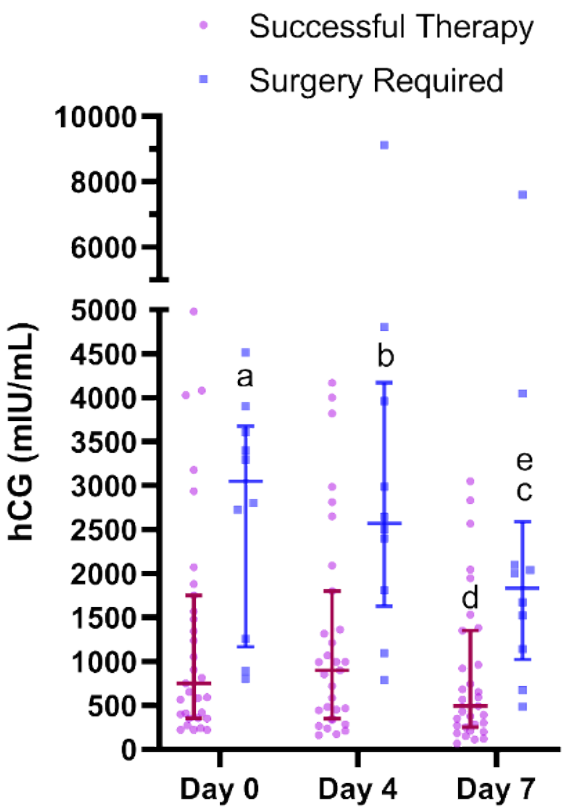

Figure 1. Median serum hCG values obtained as baseline at admission (day 0) before the administration of single-dose methotrexate (MTX) regimen and 4 and 7 days (days 4 and 7, respectively) later after MTX treatment in patients with intact tubal ectopic pregnancy whose medical treatment was successful with MTX treatment and who required tubal surgery because of abdominal hemorrhage during MTX treatment.
Data were presented as median with interquartile range in whisker plot with scatter dots representing all the measurements.
[a,b,c]Significantly different versus days 0, 4, and 7 in patients with successful MTX treatment ($p<0.05$).
[d]Significantly different versus days 0 and 4 in patients with successful MTX treatment.
[e]Significantly different versus day 4 in patients undergone tubal surgery because of abdominal hemorrhage.

DISCUSSION

In this study, we investigated the feasibility of predicting the results of a single-dose MTX regimen early by detecting serum hCG on days 0 and 4 to avoid the need for a close 7-day follow-up of patients. This may help to determine the requirement of a second-dose MTX or surgical interventions without delay in patients who will have possibly unfavorable outcomes. With respect to predicting the success of single-dose MTX treatment, decrease of HCG value on day 4, in

Table 2. Change of serum hCG values on day 4 versus day 0 in patients with intact tubal ectopic pregnancy whose medical therapy was successful with single-dose methotrexate (MTX) regimen and who required tubal surgery because of abdominal hemorrhage during MTX treatment.

Change of serum hCG	Successful treatment (n=31)	Surgery required (n=10)
Decrease (n=22)	20 (64.5%)	2 (20%)
Increase (n=20)	11 (35.5%)	8 (80.1%)

Chi-square test revealed that the rate of decrease in serum hCG values in the patients with intact tubal ectopic pregnancy whose medical therapy was successful with MTX administration compared to in patients who required tubal surgery because of abdominal hemorrhage during MTX treatment ($p<0.05$).

addition to low HCG value on day 0, was found valuable to predict treatment failure in patients with intact TEP.

In women with a non-ruptured TEP and in a clinically stable condition, intramuscular MTX regimen, laparoscopic tubal surgery as well as expectant management without interventions in a selected subset of patients are the main treatment options. Baseline clinical, laboratory, and radiologic evaluations as well as informed choice of patients are used for determination of appropriate management regimen. Use of MTX regimen is determined mainly by initial serum hCG level. The single-dose regimen may be the best option in general, when there is a relatively low initial serum hCG level or a plateau in serum hCG values, and the two-dose regimen may be preferred when initial serum hCG levels are high. When serum hCG level did not decrease by at least 15% from day 4 to day 7 after MTX administration, there is a high risk of treatment failure. In that case, patients may require additional MTX administration or surgical intervention (1). According to the NICE guideline on the diagnosis and management of early pregnancy complications including ectopic pregnancies, all women diagnosed with intact TEPs should be managed actively with MTX regimen or surgical intervention (12). Although mortalities are rare depending on the complications of ectopic pregnancies in developed countries, the burden of disease is high regarding to the costs of diagnostic work up and expensive treatment modalities requiring outpatient and impatient follow-up (13).

Nowadays, it is possible to diagnose TEP earlier with the advancements of ultrasound imaging and MTX administration has become the first-line treatment in many cases (10). Women should have their serum hCG levels monitored on a regular basis after taking MTX. The level may rise in the first few days, but it should drop by 15% to 25% by day 7; if it has not, a second dose, which is required in 15% to 20% of patients, should be taken into consideration. Most patients who get MTX treatment have abdominal cramps and pain by day 3 to 7 after treatment, and there is no consensus on predictors of effectiveness for medical therapy. When such patients arrive at the emergency room, a pelvic ultrasound should be done to check for free fluid. Hemoperitoneum may signal the treatment failure and TEP rupture, which occurs in 7 percent to 14 percent of patients treated with MTX. These patients may require a second dosage of MTX or surgical intervention, which should be discussed with the patient's gynecologist. It is possible for trophoblastic tissue to regenerate unless the damaged tube is entirely removed. To guarantee complete remission, the blood hCG level should be trended until it is undetectable, whether the patient is handled medically or surgically (3).

Most cases of TEP that are detected early can be treated successfully either with minimally invasive surgery or with medical management using MTX (1), with consideration of hemodynamic stability, wish to have a baby, the status of tubal mass (3). hCG should double every 1.4 to 2.1 days in a normal pregnancy, until reaching at more than 100,000 mIU/mL. In stable patients presenting with vaginal bleeding or abdominal pain, ultrasonography should be the next step in evaluation. An IUP should be consistently visible on transvaginal ultrasonography if the hCG level is greater than 1500 to 3510 mIU/mL (3).

Stovall et al. reported first results of the single-dose MTX regimen for medical management of TEP in 1991(14,15). Due to high success rate with a range of from 65 to 96%, MTX is still used worldwide (1,4,5,10,13,11,12,16). The change in serum hCG levels between days 4 and 7 after MTX administration is the most important predictive indicator for treatment success. Initial treatment success is defined by a decline in hCG levels of 15% or more, which permits for biological follow-up in the future. The main disadvantage of a single-dose MTX regimen is the requirement for a 7-day treatment period and multiple visits to check treatment success. Early detection of treatment progress may thus improve adherence to the regimen and reduce psychological stress, medical visits, and overall health costs (11). Yuk et al. performed systematic review and meta-analysis of single-dose and non-single-dose MTX regimens in the treatment of TEP (4). They noted that the therapeutic effects of the single-dose regimen and non-single-dose regimens were similar, but the single-dose regimen had a lower adverse effect rate compared with non-single-dose regimens. They reported that the single-dose regimen was optimal for the medical treatment of TEP although the time to resolution was longer with the single-dose regimen.

Jurkovic et al. examined the success rates of MTX against placebo for the conservative treatment of TEP (13). They found that the rates of success were similar for these treatment modalities: 83% with MTX and 76% with placebo. They noted that the serum level of hCG was the only clinical parameter found to be meaningfully related to the outcome of MTX regimen. They did not recommend the routine use of MTX for the treatment of clinically stable women diagnosed with TEP presenting with low serum hCG (<1500 mIU/L). They concluded that in women with TEP and serum hCG value ≥1500 mIU/L, MTX may offer a safe and cost-effective alternative to surgery; however, this needs confirmation with further studies. Aydın et al. tested MTX treatment at a dose of 22-40 mg/m^2 and 41-49 mg/m^2 in addition to 50 mg/m^2 in the treatment of TEP (10). They recorded the serum hCG levels on Days 0, 1, 4, and 7 and in case of a decline in serum hCG levels less than 15% on days 4 and 7, they administered a second dose of MTX was administered. In addition, irrespective of the pre-treatment serum hCG levels, the overall success rate including the success rate after the second dose of MTX was evaluated. They reported their success rates as the following: 76.5% (13/17) with the 22–40 mg/m2, 84.0% (68/81) with the 41–49 mg/m^2, and 85.7% (12/14) with the ≥50 mg/ mg/m^2 doses. They found no difference among those success rates.

Brunello et al. investigated the prognostic value of changes in the serum hCG values as the ratio of days 4/1 after single-dose MTX regimen in TEP patients (11). They found that a ratio ≤0.7 was associated with a success rate of 94% after one course of MTX and 100% after two courses. In contrast, a ratio >1.7 was associated with a failure rate of 100%. They suggested that in patients with a hCG decline ≥ 30%, a simplified follow-up may be appropriate and in those with a hCG increase >70%, second course of treatment need to be administered. Gabbur et al. evaluated whether serum hCG level on day 4 following MTX treatment in TEP patients could predict success or failure of single-dose regimen compared to that on day 7 (17). They concluded that serum hCG level on day 4 of MTX administration did not predict successful single-dose MTX treatment or the need for surgery; whereas hCG on day 7 could be used for prediction. Nguyen et al. evaluated serum hCG levels measured on days 0 and 4 to predict the success of MTX regimen in TEP patients (18). They found that serum hCG level was found as decreased on day 4 in 40% of cases, and 100% of those cases had treatment success; whereas serum hCG level was increased in 60.0% of cases, and 61.8% of those cases had treatment success. They concluded that changing trend of serum hCG value on day 4 could be used as a reliable predictor of treatment success.

Ustunyurt et al. conducted a study to predict the success of single-dose MTX regimen with the change of serum hCG value on day 4 compared to day 0 (19). They reported that the rate of decreasing serum hCG value on day 4 was significantly more in patients with successful

management compared to those with treatment failure (61.9 vs. 37.5%). They highlighted that the change of serum hCG value on day 4 compared to day 0 had a predictive value to assess the success of MTX treatment. Dai et al. reported a different follow-up strategy for serum hCG and did not used serum hCG value on day 4 (20). They noted that when serum hCG value measured on day 7 less than 50% on day 1, single dose MTX could be accepted as successful, especially in patients with a serum hCG value less than 2000 mIU/mL on day 1. They highlighted that when serum hCG value on day 1 was higher, there was no difference between conventional and new follow-up strategies. Celik et al. highlighted that a decrease of > 15% in serum hCG values from days 0 to 4 did not seem to be a better predictor of successful treatment compared to those from days 4 to 7 in patients administered single-dose MTX regimen for TEP (21). Since there is no consensus on the value of serum hCG on day 4 since no management decisions are made until obtaining serum hCG value on day 7 in the conventional single-dose MTX regimen, Atkinson et al. examined the place of alternative serum hCG follow-up omitting the serum hCG on day 4 in patients administered a second dose of MTX if the serum hCG fall was <25% between days 0 and 7 or days 1 and 7. They found no additional advantage of those new protocols (6).

Others have suggested that the Day-4 serum hCG is important because any drop between Days 1 and 4 (or Day 0–4 in some studies) is associated with a high chance of avoiding both a second dose of MTX and surgery (at least 88%, and up to 97% for a fall >20%), providing early reassurance to women (11–14). Some speculate that a drop between Days 1 and 4 could eliminate the necessity for an assay on Day 7 and free up a more flexible follow-up plan (22,23,24,25).

As seen from the pertinent literature including studies searching an optimal hCG follow-up during the administration of single-dose MTX regimen in patients with intact TEP, there are studies finding the conventional hCG evaluation is the method of choice; however, according to the findings of considerable high number of studies, the decrease of serum hCG on day 4 compared to day 0 in addition to conventional evaluation seems to be a valuable method to predict success rate of MT treatment to reduce complications and to shorten the duration of management. During management of TEP patients with MTX regimen, gynecologists also need to consider individual variations of clinical success related to pharmacogenetic properties of MTX. 5,10-methylenetetrahydrofolate reductase, a key enzyme of folate metabolism, is a competitive target of MTX polyglutamates as active metabolites of MTX, which converts 5,10-methylenetetrahydrofolate to 5,10-methyltetrahydrofolate in the folic acid cycle and then supplies methyl groups for the conversion of homocysteine to methionine, which is essential for nucleotide synthesis. Genetic variations of 5,10-methylenetetrahydrofolate reductase result in reduced activity from 30 % to 10 % of normal activity in 12% of white and Asian populations when homozygosity present and about 60% of normal activity in 40% of the population when heterozygosity present (7).

This study does, however, have significant drawbacks. The key limitations are the small sample size and retrospective nature of the study. However, despite the limited sample size, our success rates are comparable to most of the previous research published in the literature. In clinical practice, gynecologists need to consider not only the decrease rate of serum hCG from day 4 to 7 but also from day 0 to 4, with this manner, they can administer a second-dose MTX or perform a surgical intervention and prevent harmful complications of tubal ectopic pregnancy in at least a subset of patients. Further studies need to be performed to better predict success of MTX treatment in patients grouped according to clinical, ultrasonographic and individual pharmacogenetic properties of MTX.

Ethics Committee Approval: Süleymaniye Obstetrics and Gynecology Training and Research Hospital Educational Planning and Coordination Committee (EPKK) Decision (06.02.2013)
Conflicts of Interest: The authors declare that there is no conflict of interest regarding the

publication of this article.

Funding: Our research did not receive any specific grant from funding institutions.

Informed Consent: Retrospective

Author contributions: All authors contributed to the conception and design of the study, data collection, analysis or interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be published.

REFERENCES

1. [No authors listed]. ACOG Practice Bulletin No. 193: Tubal Ectopic Pregnancy. Obstet Gynecol. 2018 ;131(3):e91-e103. https://doi.org/10.1097/AOG.0000000000002560
2. Sharvill J. Ectopic pregnancy and miscarriage: how long should a woman have to wait for a scan? BMJ. 2019 Dec 31;367:l7070. https://doi.org/10.1136/bmj.l7070
3. Pontius E, Vieth JT. Complications in Early Pregnancy. Emerg Med Clin North Am. 2019 May;37(2):219-237. https://doi.org/10.1016/j.emc.2019.01.004
4. Yuk JS, Lee JH, Park WI, Ahn HS, Kim HJ. Systematic review and meta-analysis of single-dose and non-single-dose methotrexate protocols in the treatment of ectopic pregnancy. Int J Gynaecol Obstet. 2018;141(3):295-303. https://doi.org/10.1002/ijgo.12473
5. Alur-Gupta S, Cooney LG, Senapati S, Sammel MD, Barnhart KT. Two-dose versus single-dose methotrexate for treatment of ectopic pregnancy: a meta-analysis. Am J Obstet Gynecol. 2019;221(2):95-108.e2. https://doi.org/10.1016/j.ajog.2019.01.002
6. Atkinson M, Gupta S, Mcgee T. βhCG monitoring after single-dose methotrexate treatment of tubal ectopic pregnancy: is the Day 4 βhCG necessary? A retrospective cohort study. Aust N Z J Obstet Gynaecol. 2014 ;54(5):475-9. https://doi.org/10.1111/ajo.12257
7. Ulrich CM, Yasui Y, Storb R, Schubert MM, Wagner JL, Bigler J. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood. 2001 Jul 1;98(1):231-4. https://doi.org/10.1182/blood.V98.1.231
8. Deng J, Chen L, Xue H, Zeng FX, Niu PG, Shi DH. Contribution of genetic polymorphism of methylene tetrahydrofolate reductase on the effect of methotrexate in ectopic pregnancy patients. J Clin Lab Anal. 2020;34(1):e23030. https://doi.org/10.1002/jcla.23030
9. Jensen KK, Sal M, Sohaey R. Imaging of Acute Pelvic Pain: Pregnant (Ectopic and First-trimester Viability Updated). Radiol Clin North Am. 2020;58(2):347-361. https://doi.org/10.1016/j.rcl.2019.11.003
10. Aydın GA, Özgen G, Yavuz A. Comparative study of low-dose versus standard single-dose methotrexate: A novel approach for medical treatment of ectopic pregnancy. J Obstet Gynaecol Res. 2020;46(2):286-292. https://doi.org/10.1111/jog.14167
11. Brunello J, Guerby P, Cartoux C, Yazigi A, Baujat M, Parant O. Can early βhCG change and baseline progesterone level predict treatment outcome in patients receiving single dose Methotrexate protocol for tubal ectopic pregnancy? Arch Gynecol Obstet. 2019 ;299(3):741-745. https://doi.org/10.1007/s00404-019-05068-1
12. National Institute for Health and Care Excellence. Ectopic pregnancy and miscarriage: diagnosis and initial management in early pregnancy of ectopic pregnancy and miscarriage. CG154. 2012. http://publications.nice.org.uk/ectopic-pregnancy-and-miscarriage-cg154.
13. Jurkovic D, Memtsa M, Sawyer E, Donaldson AN, Jamil A, Schramm KA. Single-dose systemic methotrexate vs expectant management for treatment of tubal ectopic pregnancy: a placebo-controlled randomized trial. Ultrasound Obstet Gynecol. 2017;49(2):171-176. https://doi.org/10.1002/uog.17329
14. Stovall TG, Ling FW, Buster JE. Outpatient chemotherapy of unruptured ectopic pregnancy. Fertil Steril. 1989 ;51(3):435-8. https://doi.org/10.1016/S0015-0282(16)60549-6
15. Stovall TG, Ling FW, Gray LA. Single-dose methotrexate for treatment of ectopic pregnancy. Obstet Gynecol. 1991 ;77(5):754-7.
16. Barnhart KT, Gosman G, Ashby R, Sammel M. The medical management of ectopic pregnancy: a meta-analysis comparing "single dose" and "multidose" regimens. Obstet Gynecol. 2003 Apr;101(4):778-84. https://doi.org/10.1097/00006250-200304000-00028
17. Gabbur N, Sherer DM, Hellmann M, Abdelmalek E, Phillip P, Abulafia O. Do serum beta-human chorionic gonadotropin levels on day 4 following methotrexate treatment of patients with ectopic pregnancy predict successful single-dose therapy? Am J Perinatol. 2006 ;23(3):193-6. https://doi.org/10.1055/s-2006-934097
18. Nguyen Q, Kapitz M, Downes K, Silva C. Are early human chorionic gonadotropin levels after methotrexate therapy a predictor of response in ectopic pregnancy? Am J Obstet Gynecol. 2010;202(6):630.e1-5. https://doi.org/10.1016/j.ajog.2010.03.022
19. Ustunyurt E, Duran M, Coskun E, Ustunyurt ÖB, Simşek H. Role of initial and day 4 human chorionic

gonadotropin levels in predicting the outcome of single-dose methotrexate treatment in women with tubal ectopic pregnancy. Arch Gynecol Obstet. 2013 ;288(5):1149-5.
https://doi.org/10.1007/s00404-013-2879-8

20. Dai Y, Zhang G, Zhu L, Lang J, Liu Z. Routine β-Human Chorionic Gonadotropin Monitoring for Single-Dose Methotrexate Treatment in Ectopic Pregnancy. J Minim Invasive Gynecol. 2017 ;24(7):1195-1199.
https://doi.org/10.1016/j.jmig.2017.07.025
21. Çelik H, Tosun M, Işık Y, Avcı B. The role of early determination of β-human chorionic gonadotropin levels in predicting the success of single-dose methotrexate treatment in ectopic pregnancy. Ginekol Pol. 2016; 87(7):484-7.
https://doi.org/10.5603/GP.2016.0030
22. Bottin P, Gnisci A, Crochet P, Butzbach P, Cravello L, Gamerre M. Prognostic value of early hCG changes after methotrexate injection for ectopic pregnancy. Gynecol Obstet Fertil. 2014 ;42(1):3-7.
https://doi.org/10.1016/j.gyobfe.2013.08.006
23. Agostini A, Blanc K, Ronda I, Romain F, Capelle M, Blanc B. Prognostic value of human chorionic gonadotropin changes after methotrexate injection for ectopic pregnancy. Fertil Steril. 2007 ;88(2):504-6.
https://doi.org/10.1016/j.fertnstert.2006.11.138
24. Skubisz M, Dutton P, Duncan WC, Horne AW, Tong S. Using a decline in serum hCG between days 0-4 to predict ectopic pregnancy treatment success after single-dose methotrexate: a retrospective cohort study. BMC Pregnancy Childbirth. 2013 Feb 1;13:30.
https://doi.org/10.1186/1471-2393-13-30
25. Kanmaz AGK, Hamdi İnan A, Beyan E, Budak A. Various BhCG monitoring protocols for predicting the success of single-dose methotrexate in the treatment of ectopic pregnancy. J Obstet Gynaecol. 2019 ;39(6):811-815.
https://doi.org/10.1080/01443615.2019.1575344