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Original Article

Predictors of failure of the commonly used single-dose methotrexate protocol for treating tubal ectopic pregnancies

Eser Sefik Ozyurek [a, *], Evren Akmut [a], Erdal Kaya [a], Aysu Akca [b], Ozgur Akbayır [b]

[a] Bagcilar Research and Training Hospital, Gynecology and Obstetrics Department, Istanbul, Turkey
[b] Kanuni Sultan Suleyman Research and Training Hospital, Gynecological and Obstetrics Department, Istanbul, Turkey

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ABSTRACT

Objectives: This study identified patients who would benefit from an earlier additional medical intervention and/or continuing close surveillance even if commonly used parameters indicated sufficient medical treatment to determine markers of treatment failure.

Materials and methods: A retrospective analysis of patients with a preliminary diagnosis of ectopic pregnancy treated with the single-dose methotrexate protocol. *Group 1*: cases cured with a single dose of methotrexate; *Group 2*: cases who required more than one dose of methotrexate or surgery following the first dose. Demographics, clinical/sonographic findings, observation period, and β-human chorionic gonadotropin (hCG) levels were compared among the two groups. Thresholds were defined and a regression analysis was performed to define independent predictors of failure.

Results: Data from 120 patients were analyzed: Group 1 (n = 92); Group 2 (n = 28). β-hCG levels measured at all time points, and day (0–4) and day (4–7) changes, presence of adnexial masses, and infertility were significantly different among the two groups. Only the day (0–4) and day (4–7) changes in β-hCG levels were independent predictors of failure.

Conclusion: Day (0–4) thresholds or newly defined day (4–7) thresholds were not more sensitive than the conventional day (4–7) criteria. Day (0–4) β-hCG levels increased by more than 9.7% in half the patients who required additional methotrexate doses or surgery despite fulfillment of the conventional day (4–7) criteria. In contrast, no cases of treatment failure were observed if the day (0–4) decrease was >26.6%.

Introduction

Ectopic pregnancy is the leading cause of pregnancy-related mortality in the first trimester. An earlier diagnosis combined with numerous treatment options has decreased related mortality [1].

The prevalence of ectopic pregnancy has been increasing in the last four decades due to increasing rates of pelvic infections, smoking, infertility, and more sensitive laboratory methods and imaging technologies providing an earlier diagnosis [2].

The proportion of indeterminate cases with a later definite diagnosis has increased with more sensitive diagnostic methods [3].

The classic assessment of treatment success defined by Stovall et al. and revalidated by Kirk et al. has 88–93% sensitivity but eliminates a large number of patients as false negatives, if not a large proportion in a population with high pregnancy rates [4,5].

Our aim was to analyze independent markers of treatment failure to identify patients who would benefit from an earlier additional medical intervention and/or continuing close surveillance even if conventional parameters indicated sufficient medical treatment.

Materials and methods

This was a retrospective analysis of patients with a preliminary diagnosis of ectopic pregnancy, who applied to the Bagcilar Research and Training Hospital Obgyn Department emergency section or ambulatory clinic from September 2012 to March 2016 and who were treated with the methotrexate (Mtx) single-dose protocol defined by Stovall et al. [4]. This study was approved by

* Corresponding author. Bagcilar Research and Training Hospital, Gynecology and Obstetrics Department, Şişli Kuştepe Mahallesi Tomurcuk Street Izmen Site A1, 9/42-43, Istanbul, Turkey.
E-mail address: eozyurek@yahoo.com (E.S. Ozyurek).

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the Medical Ethics Committee (Project number: 406/2015). The patients' data were obtained from the patients' database (MEDIN 2.0.0) using the following queries: "ectopic pregnancy" as the diagnostic input. The surgical records were revised for the diagnosis of "ectopic pregnancy".

Ectopic pregnancy was a preliminary diagnosis when blood β-human chorionic gonadotropin (hCG) levels were >1500 mIU/ml and no intrauterine gestational sac was detected (examined with 5–8 MHZ transvaginal sonography) or when blood β-hCG levels were <1500 mIU/ml but the level was increasing and was <50% during the previous 48 h and blood β-hCG levels did not regress despite a uterine evacuation. Following the preliminary diagnosis of ectopic pregnancy, an observation period of 1–8 days elapsed before the first dose of Mtx. In the intervening period, the patients were regularly monitored for abnormalities in hemodynamics by measuring blood counts and blood β-hCG levels every other day. If the blood β-hCG level did not regress spontaneously (≥15%/24 h drop during serial β-hCG measurements with or without a uterine evacuation performed according to findings of endometrial thickness ≥10 mm, sonographic view of intrauterine conception or passage of tissues with uterine bleeding), medical treatment was started.

The single-dose Mtx treatment protocol was used for patients with a preliminary diagnosis of ectopic pregnancy and a β-hCG level <10,000 mIU/mL; with nonhomogeneous adnexial masses ≤5 cm; absence of fetal cardiac activity, absence of signs of hemoperitoneum or hypovolemia; and normal blood counts and renal/liver function tests. Mtx was administered at a single intramuscular (IM) dose of 50 mg/m^2 on day 0, unless there were contraindications. The patients were hospitalized and watched expectantly following the drug administration while being checked for signs of acute abdomen and serious intraabdominal bleeding until a surgical intervention was indicated or for 7 days. Blood tests (biochemistry, blood parameters, and β-hCG) were measured on day 4. If a ≥15% decrease was not observed in the blood β-hCG level from days 4–7 or if a plateauing or rising blood β-hCG level was observed during follow-up of resolution, a second dose of 50 mg/m^2 Mtx was administered IM, followed by the same cycle of follow-up as for the first Mtx dose. If a ≥15% drop was observed, the patients were observed weekly.

Failure of the single-dose Mtx treatment was considered if extra doses of methotrexate were required, or if a surgical intervention was made due to an intervening clinical picture of tubal rupture.

Exclusion criteria from the analysis included: spontaneous regression during observations with or without a uterine evacuation; lost to follow-up; nontubal ectopic pregnancy; and surgery prior to medical treatment.

Data extracted from the database included demographics and clinical presentations of the patients; sonographic findings, length of the observation period before treatment, and all β-hCG levels measured including those measured on days 0, 4, and 7 following Mtx administration. Intervening surgeries and clinical outcomes are summarized in Table 1 and Fig. 1. Data derived for analysis were the percentage changes in blood β-hCG levels: [β-hCG] ((Day 0–Day 4)/Day 0) and [β-hCG] ((Day 4–Day 7)/Day 4); also represented by [β-hCG] (Day 4/Day 0) and [β-hCG] (Day 7/Day 4), respectively; and daily changes during the observation period were derived by logarithmic transformation of the ratios. In this study, we refer to changes in β-hCG as quotients of values measured on the annotated days (e.g., the conventional day 4 to day 7 change of 15% as ([β-hCG] level on day 7)/([β-hCG] level on day 4) represented by *the day (7/4) change of 0.85*, etc.)

Univariate tests were used to define significantly different variables among the single-dose Mtx successfully *(Group 1)* cured and the single dose Mtx failed *(Group 2)* groups requiring more than one dose of Mtx or surgery after the initial dose. A receiver operating characteristics (ROC) curve analysis of these variables defined the threshold values that were used to derive new dichotomized variables for the multivariate logistic regression analysis to predict

Table 1
Characteristics of the patients who were successfully treated (Group 1) or who failed medical treatment (Group 2).

	Group 1[a] (n = 92)	Group 2[b] (n = 28)
Age	30.6 ± 0.6	30.3 ± 0.9
Gravida	2.8 ± 0.2	3.3 ± 0.3
Abortus	1.9 ± 0.2	2.2 ± 0.6
Active Contraception, n (%)	14 (15.2)	5 (17.9)
Infertile couple, n (%)[c]	7 (7.6)	6 (21.4)
Smoking, n (%)	26 (28.3)	10 (35.7)
Previous Ectopic Pregnancy, n (%)	4 (4.3)	2 (7.1)
Previous abdominopelvic operations, n (%)	38 (41,3)	10 (35,7)
History of pelvic infection, n (%)	4 (4.3)	2 (7.1)
Endometrial thickness (mm)	8.6 ± 0.5	8.1 ± 0.8
Number of cycles of single dose Mtx[c]	1	1.6 ± 0.1
Mtx Single-dosage administered (mg)	80.7 ± 0.9	81.1 ± 1.7
Watchfull period (days)	2.3 ± 0.2	1.7 ± 0.1
DailyΔ% (β-hCG) during observation[c,d]	0.4 ± 0.1	6.9 ± 0.3
Day 0:[β-hCG] of First Mtx Treatment (mIU/mL)[c]	1472 ± 167.8	2319.7 ± 375
Day 4:[β-hCG] of First Mtx Treatment (mIU/mL)[c]	1418.1 ± 202	2942.2 ± 459.4
Day 7:[β-hCG] of First Mtx Treatment (mIU/mL)[c]	814.6 ± 122	2686.3 ± 493
Day (4/0)[e,c]	0.79 ± 0.05	1.27 ± 0.05
Day (7/4)[f,c]	0.55 ± 0.06	0.86 ± 0.06
Hospitalization period (days)[c]	7.1 ± 0.3	16 ± 1.1
Missed Period/positive pregnancy test, n (%)	32 (34,7)	11 (39,3)
Pain/bleeding, n (%)	57 (61,9)	13 (46,4)
Adnexial Mass (+), n (%)[c]	29 (31.5)	21 (75)
Free peritoneal fluid, n (%)	32 (34,4)	9 (28,1)

[a] Resolved with single dose methrotrxate (Mtx).
[b] Required additional Mtx doses/surgery for tubal rupture.
[c] $P < 0.05$.
[d] Daily change in β-human chorionic gonadotropin (hCG) levels during the waiting period (%/day).
[e] Ratio of β-hCG levels on day 4 to day 0 following the first Mtx dose.
[f] Ratio of β-hCG levels on day 7 to day 4 following the first Mtx dose.

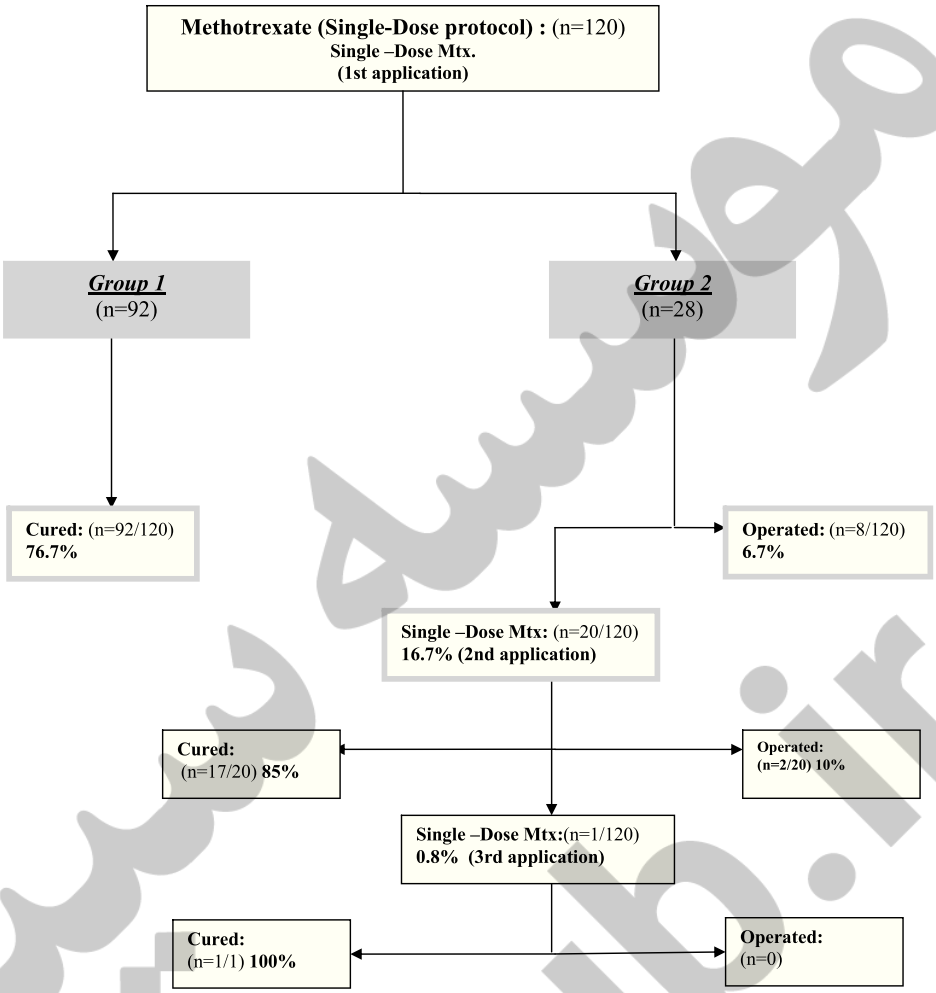

Fig. 1. Clinical progress of patients in Groups (1 and 2).

failure of the single-dose Mtx treatment. *P*-values < 0.05 were considered significant. Microsoft Excel (Microsoft Inc. Redmond, WA, USA) and SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software were used to analyze the data.

Results

A total of 278 patient data entries with a primary diagnosis of ectopic pregnancy were retrieved from the hospital database. Among these, 130 (46.8%) patients were defined as eligible for the single-dose Mtx treatment, and the complete follow-up data of 120 were retrieved, with the remainder (10 patients) being excluded due to missing medical treatment data. *The 120 patients who* received single-dose Mtx as described previously *were included in the final analysis of the study*.

The progress of medical treatment is summarized in Fig. 1.

Cumulatively, 92/120 *(76.7%)* patients *were regarded as successfully cured with the single-dose protocol (Group 1) and the remaining 28/120 (23.4%) comprising Group 2 required extra Mtx doses and/or underwent surgery*.

The β-hCG level failed to decline following the initial Mtx dose in *12 cases*, according to the conventional standard criteria of at least 15% and received a second Mtx dose. Following a satisfactory decline in the day 4–7 interval, *eight cases* received a second Mtx dose, within 11.8 ± 2 days following discharge due to plateauing or rising β-hCG levels. Eight *cases* underwent surgery due to tubal rupture after the first Mtx dose.

Of the 20 cases who received a second Mtx dose, *17/20 (85%)* ectopic pregnancies *regressed*. In addition, *2/20 (10%)* cases underwent surgery following the second single-dose Mtx; one on day 7 and the other 3 days following a favorable response to Mtx due to a tubal rupture. Finally, only one patient required a third Mtx dose, and this case resolved. Overall, 110/120 (91.7%) (92 following the 1st dose; 17 following the 2nd dose, and one following the 3rd dose of Mtx) of all ectopic pregnancies were managed medically without surgery for tubal rupture.

The patient characteristics and the significantly different variables among Groups 1 and 2 as defined in the univariate analysis are summarized in Table 1. The day 0, 4, and 7 blood β-hCG levels; the day (4/0), the day (7/4) changes in the β-hCG levels, the daily changes during the observation period before medical treatment, and the rates of infertility and positive adnexial findings were significantly different between Groups 1 and 2.

Of these variables, *the day 0 β-hCG level* (β-hCG levels 0, 4, and 7 days following Mtx treatment were strongly correlated in both groups), the *day (4/0)*, and *day (7/4) changes* were analyzed by the ROC with respect to the single dose treatment outcome (Fig. 2 and Table 2). Threshold values with optimum sensitivity and specificity were defined for day 0 β-hCG levels, the day (4/0) and the day (7/4) changes of 1070 mIU/mL, 1.097 and 0.76, respectively. Using the

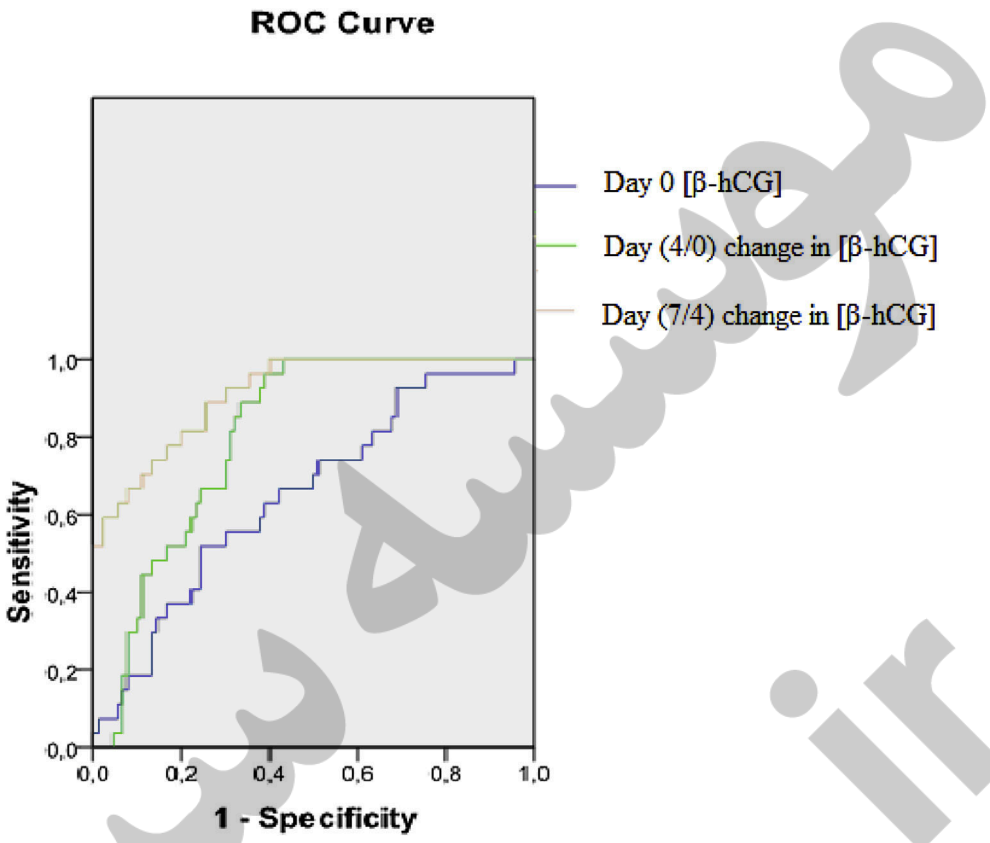

Fig. 2. Receiver operating characteristics (ROC) curve analysis of the significantly different variables.

Table 2
Optimum thresholds for the significantly different variables.

	AUC	Optimum threshold	Sensitivity (%)	Specificity (%)
D0 beta hCG mIU/mL	0,65	1070	62	64
Day (4/0)[a]	0,79	1.097	71	70
Day (7/4)[b]	0,91	0.76	81	79

[a] Ratio of β-human chorionic gonadotropin (hCG) levels on day 4 to day 0 following the first methotrexate (Mtx) dose.
[b] Ratio of β-hCG levels on day 7 to day 4 following the first Mtx Dose.

newly defined day (4/0) criteria (1.097) in addition to the conventional day (7/4) criteria (0.85), 56.3 [29.5–74.8]% of the false negatives missed were detected using only the conventional day (7/4) conventional criteria.

Dichotomous variables were produced for each case with respect to the threshold values of these three numerical variables. Then, a logistic regression analysis was performed for the variables that were significantly different in the univariate analysis. The binary logistic regression model was significant ($P < 0.001$) with a Nagelkerke R square value of 0.5 and a predictive value for the model of 75.6%. The results of the analysis are summarized in Table 3.

The predictive values of the thresholds defined for the day (4/0) (1.097), day (7/4) (0.76), and the conventionally used day (7/4) threshold (0.85) are summarized in Table 4.

Discussion

In this study, the only *independent determinants of treatment failure* which were significantly different between the successful and failed single-dose Mtx protocol treatment groups in patients with a tubal ectopic pregnancy were the Day (4/0) change threshold of (1.097) and the day (7/4) change threshold of (0.76). The sensitivity, specificity, positive and negative predictive values (PPV-NPV) for the Day (4/0) (i.e.:1.097) and the Day (7/4) (i.e.:0.76) threshold values were similar. The NPV and sensitivity of these two defined thresholds to predict failure were both similar to the conventionally used Day (7/4) threshold of 0.85. Hence, the calculated optimum Day (4/0) and the Day (7/4) thresholds predicted treatment failure of the single-dose Mtx protocol as accurately as the conventional criteria, but at an earlier stage. In this study, it is also suggested that the proportion of cases falsely decided as 'sufficiently treated' by using the conventional criteria may be decreased by half by using the earlier day (4/0) threshold criteria.

Table 3
Logistic regression analysis to define independent predictors of single-dose methotrexate (Mtx) treatment failure.

	B	S.E.	Sig.	Odds ratio	95% C.I. for odds ratio	
					Lower	Upper
Day 0 βhCG >1070mIU/mL	NS	NS	NS	NS	–	–
Day (4/0) [βhCG] < 1.097%[a]	*1.58*	*0.57*	*0.005*	*4.9*	*1.6*	*14.6*
Day (7/4) [βhCG] < 0.76%[b]	*2.3*	*0.86*	*<0.001*	*10*	*3.3*	*30.5*
Adnexial mass (+)	NS	NS	NS	NS	–	–
Daily %Change during the observation period	NS	NS	NS	NS	–	–
Infertility	NS	NS	NS	NS	–	–
Constant	−1.373	0.52	0.008	–	–	–

Italics: notating the variables with a significant effect in the model.
[a] Ratio of β-human chorionic gonadotropin (hCG) levels on day 4 to day 0 following the first Mtx dose.
[b] Ratio of β-hCG levels on day 7 to day 4 following the first Mtx dose.

Table 4
Predictive values of the defined Day (4–7), Day (0–4) change criteria in blood β-human chorionic gonadotropin (hCG) levels; and the conventional Day (4–7) criteria for cure (>15% drop) summarized.

	Cured with single-dose Mtx		Failed with single-dose Mtx		
	n	%	N	%	
[β-hCG] (Day 7/Day 4)[a]					
>0.76	13	14.1	*18*	*64.3*	*PPV:58 [39.3-74.9]%*
≤0.76	*79*	*85.9*	10	35.7	*NPV:88.8 [79.8-94.1]%*
Total	*92*		*28*		
	Specificity: 85.9 [76.7-92]%		*Sensitivity: 64.3 [44.1-80.7]%*		
[β-hCG] (Day 4/Day 0)[b]					
>1.097	27	*28.6*	*20*	*71.4*	*PPV:42.6 [28.6-57.7]%*
≤1.097	*65*	*71.4*	8	*28.6*	*NPV:89 [79-94.8]%*
Total	*92*		*28*		
	Specificity: 70.6 [51.1–86]%		*Sensitivity: 71.4 [51.1–86]%*		
[β-hCG] (Day 7/Day 4)[c]					
>0.85	0	–	*12*	*42,9*	-
≤0.85	*92*	–	16	*57,1*	*NPV:85,2 [76,8–91]%*
Total	*92*		*28*		
	Specificity: –		*Sensitivity: 42,9 [25–62,6]%*		

PPV: positive predictive value.
NPV: negative predictive value.
[a] Threshold ratio of blood β-hCG concentration on day 7 to day 4; equal to a change of −24%.
[b] Threshold ratio of blood β-hCG concentration on day 4 to day 0; equal to a change of +9.7%.
[c] The conventionally used ratio of blood β-hCG concentration on day 4 to day 7 equal to a change of −15%.

This may also provide an opportunity to administer an additional Mtx dose at an earlier stage which may potentially improve medical treatment success.

Ectopic pregnancy encompasses 1–2% of all pregnancies and is the cause of 4.9% of pregnancy-related mortalities [2]. Ectopic pregnancy is also the most important cause of death in the first trimester and a cause for infertility and serious morbidity; therefore, it demands an accurate and quick diagnosis [6].

Infertility, lack of contraception, larger adnexial mass sizes, higher parity, and previous tubal damage (surgery, infection) have been reported to be associated with ectopic pregnancy-related tubal rupture [7,8]. Tubal ectopic pregnancies are reported to have rupture rates of 32%, necessitating a surgical intervention. The highest risk for rupture is within the first 48 h (5–7%) following the initial diagnosis and is 2–3% for each succeeding 48 h [9].

In cases of infertility, ectopic pregnancies are diagnosed at earlier stages and with relatively high prevalences due to closer monitoring during the early pregnancy period. Moreover, closer and earlier monitoring with serial blood β-hCG levels lead to lower morbidity and mortality in this subgroup [10].

The management criteria during the early observation period before the decision for medical treatment (i.e.: uterine evacuation for all cases with abnormal trends in blood levels or selective use of uterine evacuation; duration of observation) are not well defined [11]. Uterine evacuation was performed for 25 cases at the early stage of management, with indications of suspicious findings of missed abortion, vaginal passage of tissues with blood, and excessive bleeding. In 16% (4/25) of our cases, the hormone levels failed to regress following uterine evacuation and were managed as probable ectopic pregnancies.

β-hCG monitoring is a more effective method for predicting treatment failure compared with other risk markers [12]. The daily changes in β-hCG levels during the pretreatment stage differed among the success (Group 1) and failure groups (Group 2) in our study (0.4 ± 0.1%/day vs. 6.9 ± 0.3%/day). Still, however; this variable was not found to be an independent factor for predicting treatment outcome. In contrast, Soarez et al. reported that cases of treatment failure in a group of ectopic pregnancies observed for 48 h prior to medical treatment had lower decreases in β-hCG levels [13].

Therapeutic intervention is required for a tubal ectopic pregnancy unless the β-hCG level is <1000 mIU/ml, spontaneously regressing, and there are no signs of a tubal rupture caused by the conception located in the tube(s). Otherwise, expectant management has a similar outcome as Mtx treatment [14].

Single-dose Mtx is the most common protocol used to treat ectopic pregnancies in 66–94% of patients [5,15].

The American Society's for Reproductive Medicine definition for medical treatment failure is severe abdominal pain or hemodynamic instability necessitating a surgical intervention and persistently rising (>53%/48 h) β-hCG level despite two single-dose Mtx treatments [16]. Our definition of failure included a requirement of more than one dose of Mtx or the need for a surgical intervention.

The success rate reported in previous studies with a single Mtx dose is 67–94%, with about 15% requiring more than one dose of Mtx [15,17]. Multiple doses of Mtx provide better treatment success (odds ratio = 4.75), but with higher rates of side effects [18]. Multiple doses are mostly used for atypically located ectopic pregnancies and cases showing higher risk profiles.

The conventional monitoring criteria of day (7/4) (≤0.85) to decide whether to administer another Mtx dose has disadvantages: it has a considerable false negative failure rate to predict a cure; it requires 7 days to reach a conclusion during which tubal rupture risk is higher; and patient compliance may be low [19]. Various studies (Lipscomb et al.; Nguyen et al.; Scubicz et al.; Agostini et al.) have analyzed if earlier markers could be used to predict outcomes and most focusing on producing PPVs for treatment success with thresholds of −20% to 0% [4,12,19–22].

In our study, we defined *single-dose treatment failure* as the outcome variable and calculated the sensitivity of the optimum day (4/0) threshold (1.097), as *71.4 [51.1–86]%*. We also observed that patients were considered cured if *the day (4/0) change was <0.74*.

Nowak-Markwitz et al. suggested that the initial β-hCG level is specifically associated with the need for additional Mtx doses, whereas early β-hCG *changes* are related to the risk of tubal rupture. Factors including fetal cardiac activity associated with the viability of the conceptus and progression of an ectopic pregnancy are more likely to be related to treatment failure [23].

Despite previous reports about the initial β-hCG level being a significant predictor of treatment success with reported significant

threshold levels of 2000–5000 mIU/mL, it was not an independent predictor in our study due to the low β-hCG levels of our cases, exclusion of patients who underwent surgery prior to the first Mtx dose, and variations among the definitions for treatment success [24].

This study has a few weaknesses that need to be addressed. The retrospective nature of this analysis may be a cause for selection bias. The patients' data were produced from clinical records of our Obstetrics and Gynecology department. We used logarithmically transformed data to calculate the daily β-hCG changes during the observation period.

We conclude that the defined day (4/0) and day (7/4) thresholds were independent predictors of the single-dose Mtx treatment failure. The day (4/0) threshold of (1.097) *or* the newly defined day (7/4) threshold (0.76) did not significantly increase the predictive performance of the conventional day (7/4) threshold (0.85). The day (4/0) threshold can safely be considered as an earlier marker of medical treatment failure. Using this threshold may decrease the proportion of false negative cases observed with the conventional day (7/4) threshold of (0.85) by *56.3 [29.5–74.8]%*; hence it can also be used as a supplementary criteria as well as an earlier one. We recommend closer follow-up for cases with a suboptimal day (4/0) decline even if they are considered to be favorably responding with a day (7/4) change of ≤0.85. On the contrary, if the day (4/0) change is lower than 0.74, the condition can be considered "most probably cured", as we did not observe any treatment failure below this level.

It needs to be tested in prospective randomized studies if taking the day (4/0) β-hCG responses into account in the Single Dose Mtx treatment protocol of tubal ectopic pregnancies (to apply an additional Mtx dose or prolonged close follow-up) would improve medical treatment success (and/or) provide a better standard for patient safety.

Conflicts of interest

The authors of this study have no conflicts of interest regarding this study.

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