

Human Chorionic Gonadotropin Levels 0/1-4 Days After Methotrexate Administration For Predicting Ectopic Pregnancy Treatment Success

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ABSTRACT

Ectopic pregnancy is one of the major causes of morbidity and mortality, accounting for 1 to 2% of all pregnancies. With the introduction of recent developments in diagnostic tools, the success rate of medical treatment has increased. The treatment is considered successful, if there is a 15% decline in serum human chorionic gonadotropin (hCG) levels between Days 4 and 7 of follow up in patients receiving single-dose methotrexate (MTX) treatment. In the present study, we aimed to determine whether the change in hCG levels between Days 0 and 4 is useful in predicting treatment success in patients receiving single-dose MTX protocol. A total of 93 patients who were diagnosed with ectopic pregnancy in our clinic between September 2016 and September 2018 and treated with MTX were retrospectively analyzed. Changes in hCG levels between Days 0/1-4 and 4-7 were calculated. The overall success rate of single-dose MTX treatment was found to be 71.6%. A total of 50 patients of the success group had decreased hCG levels on Day 4, while only 18 patients of the success group had increased hCG levels on Day 4. Using the cut-off value for hCG change between Days 1 and 4, a decrease of 16.1% in hCG levels was found to be the strongest predictor of treatment success. The sensitivity, specificity, and positive predictive value of this cut-off value were 89.7%, 64%, and 87.1%, respectively. In conclusion, early changes in hCG levels may be useful in the follow-up of the treatment success.

Key Words: Ectopic pregnancy, human chorionic gonadotropin, methotrexate

Introduction

Ectopic pregnancy is defined as the implantation of a fertilized ovum outside the endometrium (1,2). It accounts for 1 to 2% of all pregnancies (1-3). Besides being an important cause of morbidity, it is responsible for 3 to 4% of pregnancy-related death events (1,2). It may also cause long-term health problems, such as infertility and recurrent ectopic pregnancies (4).

Recent improvements in diagnostic methods and monitoring of serum human chorionic gonadotropin (hCG) levels, particularly have allowed early recognition of ectopic pregnancy, thus reducing morbidity and mortality (1,5). Currently, since the diagnosis of ectopic pregnancy can be made earlier, medical treatment has become the first-line treatment in many cases. Medical treatment has been shown to be effective and safe in ectopic pregnancy (6,7). In recent years, methotrexate (MTX) has been increasingly used as the main chemotherapeutic agent in the treatment of ectopic pregnancy.

For the monitoring of single-dose MTX treatment in ectopic pregnancy, the decline in serum hCG levels from Day 4 to 7 of treatment is calculated using the method described by Stovall et al. (8) which was then validated by Kirk et al. (9). In case of a >15% decline in hCG levels, the positive predictive value (PPV) of the treatment success was found to be 93%. In the literature, the success rate of the single-dose MTX treatment was reported between 65 and 95%, and the need for a second dose of MTX was found to be 3 to 27% during the treatment period (10-13). However, there are some drawbacks to waiting until Day 7 to evaluate the success of single-dose MTX treatment. Waiting until Day 7 may result in an increased risk of rupture in ectopic pregnancy which is eventually associated with a delay in the application of additional treatment doses in case of treatment failure. In addition, prolonged delays may lead to an increase in the anxiety level of the patient and reduced treatment compliance.

In the study of Skubitz et al. (14), a success rate of 85% was found in case of a decline in hCG

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Received: 09.12.2019, Accepted: 25.01.2020

levels from Day 0 to day 4, which is similar to the 15% decline in hCG levels from Day 4 to 7 used in single-dose treatment follow-up. Some recent studies have also demonstrated that a decline in hCG levels between Days 0/1-4 is useful in the evaluation of treatment success (6,14,15).

In the present study, we aimed to investigate whether the change in hCG levels from baseline to Day 4 of treatment is useful in predicting treatment success in patients receiving single-dose MTX treatment for ectopic pregnancy and to calculate an optimal cut-off value for predicting treatment success.

Materials and Methods

This retrospective study included a total of 93 patients who were hospitalized due to ectopic pregnancy and received single-dose MTX at obstetrics and gynecology clinic between September 2016 and September 2018. Inclusion criteria were as follows: hemodynamic stability without renal, hepatic, or hematological impairment, having no free fluid in the pelvic region on transvaginal ultrasound (TVUS), and having no embryonic cardiac activity. A written informed consent was obtained from each patient. The study protocol was approved by the local Ethics Committee (2011-KAEK-25 2019/08-12). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data including demographic characteristics of the patients, previous history of ectopic pregnancy, previous gynecological surgeries, the use of *in vitro* fertilization techniques, TVUS findings, beta-hCG (β -hCG) levels, pre-treatment endometrial sampling, and the need for a surgical intervention during follow-up were retrieved from the digital medical records of the hospital.

The diagnosis of ectopic pregnancy was based on the absence of an intrauterine pregnancy and on the demonstration of tubal ectopic pregnancy, when a gestational sac with a yolk sac, or embryo, or both was noted in the adnexa on TVUS together with abnormal hCG levels. In the definition of abnormal hCG levels, the pattern of rise or fall in hCG after 48 hours is considered: an increase less than 66% or a decrease more than 15% (16-19).

Pregnancy of unknown location (PUL) was defined as an empty endometrial cavity on TVUS, despite positive pregnancy test with a hCG level of 1,500 to 2,000 mIU/mL (known as the discriminatory zone). In patients having an

abnormal increase in the hCG levels without an intrauterine gestational sac or extra-uterine ectopic focus as visualized by TVUS, an endometrial sampling was performed using the Karman's cannula before treatment for failed intrauterine pregnancy (19). In our study, the diagnosis of ectopic pregnancy was established based on a decline in hCG levels less than 15 to 20% one day after endometrial sampling in patients with PUL and hCG abnormality. Of note, patients who were diagnosed with cervical and scar pregnancy were excluded from the study. Those who had fetal cardiac activity and ectopic pregnancy on TVUS and those with hCG levels above 10,000 mIU/mL were also excluded.

In the practice of obstetrics and gynecology, single-, double-, and multiple-dose MTX regimens can be used in the medical treatment of ectopic pregnancy. In our clinic, however, single-dose MTX protocol is routinely applied. According to the protocol, the body surface area (BSA) was calculated using the following formula:

$$\text{BSA (m}^2\text{)} = [\text{height (cm)} \times \text{weight (kg)} / 3600]^{1/2}.$$

In the single-dose MTX treatment regimen, 50 mg/m² MTX was administered. Pre-treatment levels of serum hCG, complete blood count, blood urea nitrogen, serum creatinine, alanine aminotransferase, and aspartate aminotransferase levels were measured and blood typing was performed. The hCG levels on Day 0/1 and on Days 4 and 7 were recorded (8,20). In case of a decline in hCG levels less than 15% on Days 4 and 7, the second dose of MTX was administered $[(\text{Day 4} - \text{Day 7 serum hCG} / \text{Day 4 serum hCG}) \times 100]$. In this study, we calculated the percentage (%) change in hCG levels between Day 0/1 (baseline) and Day 4 in predicting the treatment success in patients receiving single-dose MTX treatment using the following formula: $[(\text{Day 0/1} - \text{Day 4 serum hCG}) / (\text{Day 0/1 serum hCG})] \times 100$.

All patients who responded to treatment were followed on a weekly basis and their hCG levels were recorded, until the target hCG level (<15 mIU/mL) was achieved. Treatment failure was defined as the need for surgery during medical treatment and the requirement of a second-dose of MTX. Surgery was indicated for ruptures or an increased severity of clinical symptoms, such as pelvic pain.

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were

expressed in mean \pm standard deviation (SD), median (min-max), or number and frequency. The Mann-Whitney U test was used to compare the numerical variables between the success group and failure group in terms of % change between Day 0/1-4 and % change between Days 4 and 7. The Wilcoxon test was used to analyze the significance of % changes between Day 0/1-4 and % changes between Days 4 and 7 in success and failure groups, separately. Also, the success of the % change in hCG levels between Days 0/1-4 in distinguishing the success and failure groups and the most optimal cut-off value for the most accurate discrimination were determined by the receiver operating characteristic (ROC) curve analysis with area under the curve (AUC) values. A *p* value of <0.05 was considered statistically significant.

Results

Of a total of 93 patients diagnosed with ectopic pregnancy and treated with MTX, the % change in hCG levels between Days 0/1-4 was calculated in all patients, while the % change between Days 4 and 7 was calculated in 83 of the patients. The success group consisted of patients who achieved treatment success with a single-dose MTX administration, while the patients who required a second dose of MTX or who underwent surgery constituted the failure group. Single-dose MTX treatment was successful in 68 (71.6%) of the patients, whereas 14 patients (14.7%) received second-dose MTX and 13 patients (13.7%) underwent a surgical intervention.

The patients were divided into two groups according to the change in the hCG levels on Day 0/1 (baseline) and Day 4 (Δ Day 0/1-4): those who had decreased hCG levels on Day 4 and those who had increased hCG levels on Day 4. A total of 50 patients (73.5%) of the success group had decreased hCG levels on Day 4, while only 18 patients (26.5%) of the success group had increased hCG levels on Day 4, indicating a statistically significantly higher treatment success rate in the patients who experienced a decline in the hCG levels on Day 4 (Table 1) ($p<0.001$).

According to the % change in hCG levels between Days 4 and 7, serum hCG levels decreased by 25.7% and 46.1% on Day 7 compared to baseline in the successful treatment and treatment failure groups, respectively ($p=0.004$). According to the % change in serum hCG levels between Days 0/1-4, the hCG levels increased by 27.41% in the failure group ($n=25$) and decreased by 18.28% in

the success group ($n=68$) on Day 4 compared to baseline, indicating a statistically significant difference in the % change between Days 0/1-4 between the groups ($p=0.001$). The mean changes in hCG levels in the success and failure groups are shown in Table 2.

Furthermore, a cut-off value was estimated to distinguish between the successful and failed treatment according to the % change in hCG levels between Days 0/1-4. The change in hCG levels between Days 0/1-4 was found to be significant in distinguishing the success and failure groups (AUC=0.851, $p=0.001$). The most optimal cut-off value was found to be minimum 16.1% decrease in Day 4 to distinguish the success and failure groups. Using a cut-off value of -16.2% with an increased rate by $\geq -16.2\%$ on Day 4, It can be said that when the hCG level were decreased in forth day 16.2% or more, treatment success could be distinguished with 89.7% sensitivity and treatment failure could be distinguished with 68% specificity.(figure 1)

The PPV and negative predictive value (NPV) were found to be 89% and 68%, respectively.

Discussion

Stovall et al. (21) first introduced the use of MTX in the treatment of ectopic pregnancy in 1989 and, then, the single-dose MTX protocol was developed in 1991 (8). Currently, MTX administration is accepted as an effective and safe method in the treatment of ectopic pregnancy. For the evaluation of treatment success, a 15% decline in the hCG levels between Days 4 and 7 is considered. However, the most optimal method to determine the success of single-dose MTX treatment is still unclear. In the pre-treatment period, history of ectopic pregnancy and hCG levels are the main factors for predicting treatment success (22), while change in hCG levels is an indicator during the treatment process. It is of utmost importance to predict the treatment success earlier to determine the need for a second-dose MTX and/or urgent surgery. Early detection of resistant cases is important to initiate appropriate treatment. Early detection of treatment success has the advantage of less frequent follow-up and helps provide more precise information about treatment options and prognosis.

Recently, several studies have been conducted to determine whether early changes in the hCG levels are useful in the follow-up of treatment. In a study including 30 patients, Nguyen et al. (20) examined

Table 1. Increase and decrease in hCG levels between Days 0/1-4 according to treatment success

	Δ hCG Day 0/1-4				Total n
	Decline on Day 4		Increase on Day 4		
	n	%	n	%	
Failure group (second-dose MTX and/or surgery)	5	20.0	20	80.0	25
Success group	50	73.5	18	26.5	68
Total	55		38		93

hCG, human chorionic gonadotropin; MTX, methotrexate

Table 2. Percent change (%) in hCG levels between Days 0/1-4 and Days 4-7 in the success and failure groups

	Failure group (second-dose MTX and/or surgery)			Success group (single-dose MTX)		
	n	mean	SD	n	mean	SD
% change in hCG levels between Days 0/1-4	25	-27.41	34.00	68	18.28	29.68
% change in hCG levels between Days 4-7	15	25.68	32.17	68	46.10	21.80

hCG, human chorionic gonadotropin; MTX, methotrexate; SD, standard deviation

hCG levels between Days 0 and 4 as a predictor of MTX treatment success for ectopic pregnancy. They found that hCG levels decreased between Days 0 and 4 in 40.0% of patients, all of whom had treatment success, while hCG levels increased in 60% of patients, and 62% of these patients had treatment success. The authors reported an overall treatment success of 76.7%.

In the study of Agostini et al. (23), the most important predictor of the success of the single-dose MTX treatment was found to be a 20% decline in hCG levels between Days 1 and 4. They found that a 20% decline in hCG levels between Days 1 and 4 during single-dose MTX treatment with a sensitivity of 0.60, a specificity of 0.92, a PPV of 0.97, and a NPV of 0.35. The authors reported the need for second-dose MTX or surgical intervention in 3% of patients who experienced more than 20% decline between Days 1 and 4. As a result, they suggested that if there was a decline in hCG levels more than 20%, a less constraining schedule would be required, while if there was a decline less than 20%, routine follow-up schedule should be implemented. However, in this study, treatment failure was defined as the need for a third-dose MTX or surgery. Similarly, Bottin et al. (24) reported a 100% treatment success rate, if there was more than 20% decline in hCG levels on Day 4, compared to baseline. In addition, Levin et al. (6) found a 22% decline in hCG levels between Days 1 and 4 as the optimal cut-off value for changes in hCG.

In a study including 162 patients, Skubisz et al. (25) reported 88% treatment success in patients

with a decline in hCG levels between Days 1-4. Despite an increase in the hCG level, treatment success was achieved in 42% of the patients. In another study, a total of 206 ectopic pregnancy patients with a hCG level of less than 3,000 mg/dL were included (14). In this study, 110 patients had decreased hCG levels between Days 0 and 4 and 94 of them achieved treatment success (sensitivity 64%, specificity 73%, and PPV 85% [95% CI: 79%-92%]). Of 96 patients with increased hCG levels after treatment, 53 (55%) achieved treatment success. When the decline in hCG levels between Days 4 and 7 was examined, 136 of 206 patients had a decline more than 15% (PPV 89% [95% CI: 84-94], sensitivity 83%, and specificity 75%). If the cut-off value for hCG decline between Days 0-4 was accepted as $\geq 20\%$, the PPV of the treatment success increased to 94%, with 93% specificity and 40% sensitivity, providing prognostic information in 29% of the patients. However, there was no statistically significant difference between the protocols in predicting the success of single-dose MTX treatment ($p=0.13$). This finding indicates that any decline in hCG levels between Days 0 and 4 can predict the success of the treatment in 85% patients, being an early indicator of prognosis.

In a study of 217 patients by Kanmaz et al. (26), the role of different treatment protocols in predicting treatment success was examined. In this study, the authors found that a 15% decline in hCG levels between Days 4 and 7 was the most useful protocol for predicting treatment success. Similar to the previous studies in the literature, a

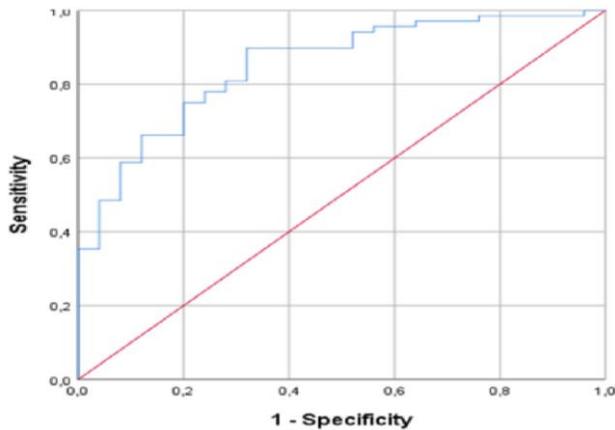


Fig. 1. ROC curve for % of changes in hCG levels between Days 0/1-4. hCG, human chorionic gonadotropin; ROC, receiver operating characteristic

21% decline in hCG levels between Days 1 and 4, the PPV and specificity were 91.5% and 91.4%, respectively (8,14,15,20,23). In another study comparing different treatment follow-up protocols in 96 patients, Sukur et al. (27) found sensitivity, specificity, PPV, and NPV as 91%, 80%, 98.8%, and 33.3%, respectively with a 15% decline between Days 4 and 7. These values were 52%, 100%, 100%, and 10.4%, respectively with a 20% decline between Days 0/1-4. The authors also found these values to be 69%, 100%, 100%, and 15%, respectively in patients with any decline in hCG levels between Days 0/1-4. According to these results, the decline in hCG levels between Days 0/1-7 had similar success rates to a 15% decline between Days 4-7 (index protocol); however, the authors found that other protocols had high false-negative rates and that more patients needed a second-dose MTX. In their study including 48 patients, Girija et al. (28) found that the most appropriate cut-off value for hCG decline between Days 0 and 4 was 10% for the evaluation of the success of single-dose MTX treatment. The PPV was calculated as 77% and the NPV as 81%. In this study, the authors reported a sensitivity and specificity of 81.8% and 50%, respectively in case of a 16.1% increase in hCG levels between Days 0 and 4. In another study including 102 patients, Wong et al. (15) found a PPV of 96%, when there was a $\geq 6\%$ decline between Days 0 and 4. In our study, the success rate of single-dose MTX was found to be 71.6%. While 73.5% of the patients in the success group experienced a decline in hCG levels on Day 4, only 26.5% of those in the success group experienced an increase in hCG levels on Day 4, compared to baseline. In addition, we calculated a cut-off value to distinguish treatment success and failure (% change) between Days 1 and 4. Similar

to the results of Girija et al., we found 89.7% sensitivity, 64% specificity, and 87.1% PPV.

In a study, an increase in hCG levels was observed on Day 4 in 50 to 70% of patients who received single-dose MTX treatment; however, treatment success was achieved in 65 to 96% of patients (29). Mashiach et al. (29) examined whether there was a relationship between the increase in hCG levels on Days 0 and 4 and the treatment success. In this study, they divided the patients who experienced hCG increase after a single-dose MTX administration as those with an increase of $\leq 50\%$ and those with an increase of $>50\%$. The risk of treatment failure significantly increased, particularly in patients with $>50\%$ increase in hCG levels (34.5%). Although surgery decisions cannot be made based solely on the increase in hCG levels, this finding may help to make decisions in controversial cases.

Furthermore, some authors have reported that hCG change between Days 0 and 4 is useful in the treatment follow-up, while some others have reported otherwise. Gabbur et al. (30) found that hCG level on Day 4 was not useful in predicting treatment success, whereas Cohen et al. (31) reported that hCG level neither on Day 4 nor the change in hCG levels between Days 0-4 were useful in predicting treatment success.

The main limitations of the present study are its retrospective design and relatively small sample size.

In conclusion, our study results suggest that early changes in hCG levels may be useful in the follow-up of the treatment success. Nonetheless, further large-scale, prospective studies are needed to confirm these findings.

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