Predictors of Success of Methotrexate in the Treatment of Ectopic Pregnancy: A New Perspective

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Objectives: Methotrexate is commonly used in the treatment of ectopic pregnancy. Outcomes with a suboptimal drop in human chorionic gonadotrophic (hCG) level are unknown. This study aimed to determine the optimal cutoff value for hCG drop in detecting treatment success, to investigate the predictors of success, and to evaluate outcome following a single dose of methotrexate as treatment of ectopic pregnancy.
Methods: A retrospective study was conducted of 182 patients with ectopic pregnancy treated with methotrexate. Outcomes included resolution of hCG or further surgical intervention. The optimal cutoff value for hCG drop in prediction of treatment success was evaluated using receiver operating characteristic curve analysis.
Results: The success rate was 79.1%. The cutoff value for hCG drop between day 4 and day 7 for prediction of success following a single dose of methotrexate was 3.34%, with a positive predictive value of 91.67%. Compared with subjects with initial hCG level of <1000 IU/L, there was a significant reduction in success for those with initial hCG levels ranging from 1000 to 3999 IU/L (odds ratio=0.184; p=0.02), 4000 to 4999 IU/L (odds ratio=0.116; p=0.03), and ≥5000 IU/L (odds ratio=0.057; p=0.01).
Conclusions: Pretreatment hCG level and hCG drop between day 4 and day 7 are good predictors of success following methotrexate treatment. With a high positive predictive value for success, conservative management with serial hCG monitoring may be considered when drop in hCG level between day 4 and day 7 is ≥3.34%.
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Introduction

Ectopic pregnancy is potentially life-threatening with an estimated incidence of 1% to 2% of all pregnancies[1]. With the wide availability of transvaginal ultrasound and quantitative serum beta–human chorionic gonadotropin (hCG) assay, ectopic pregnancies can be diagnosed early, leading to successful management without resort to surgery.

Medical treatment with methotrexate was established in the late 1980s[2]. It is an alternative to surgery and has been proven to be safe and effective[3]. Methotrexate is a folic acid antagonist that interferes with DNA synthesis and cell proliferation. Tissues with a rapid cellular turnover, such as trophoblasts, are most susceptible to its action.

Methotrexate is commonly given as a single intramuscular injection at a dose of 50 mg/m^2 body surface area according to the single-dose protocol[4]. Successful treatment in this protocol is defined by a ≥15% decrease in hCG level between day 4 and day 7 after methotrexate administration. A prospective study has found that a 15% decrease in hCG level between day 4 and 7 was a good indicator of success with positive predictive value (PPV) up to 93%[5].

Appropriate patient selection is important for methotrexate treatment success. Success rates have been reported to range from 63% to 97.6%[6]. Although associated with the initial hCG level, there is no consensus on the threshold of hCG above which methotrexate is contra-indicated. A systematic review of several observational studies reported a failure rate of ≥14.3% with single-dose methotrexate when pretreatment hCG level was >5000 IU/L, compared with a 3.7% failure rate for hCG level of <5000 IU/L[7].

The single-dose methotrexate protocol stipulated that when there was a <15% decline in hCG level from day

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4 to day 7, a second injection of methotrexate would be given[4]. How this algorithm was developed was not clearly demonstrated in the article. To the best of our knowledge, no study has evaluated the outcome after a single dose of methotrexate when the drop in hCG level between day 4 and day 7 is <15%.

This study aimed: (i) to determine the optimal cutoff value of hCG drop in detecting treatment success and avoiding the need for second methotrexate dose or surgical intervention; (ii) to identify predictors of success after a single dose of methotrexate in the treatment of ectopic pregnancy; and (iii) to evaluate the outcome following a single dose of methotrexate.

Methods

Study Design and Setting

We conducted a retrospective cohort study in a tertiary hospital in Hong Kong. Patients with ectopic pregnancy managed medically with methotrexate from January 2008 to December 2013 were included. Patients with ectopic pregnancy treated with methotrexate were identified from our hospital Gynaecology Audit Database and cross-checked with the Hospital Authority Clinical Data Analysis and Reporting System. Individual clinical records were reviewed. Ethics approval was obtained from the Kowloon West Cluster Research Ethics Committee of the Hospital Authority.

Subjects

Patients with suspected ectopic pregnancy were evaluated by transvaginal ultrasound and serial measurements of serum hCG concentration measured by enzyme immunoassay (UniCel DxI 800 immunoassay system; Beckman Coulter, US). The diagnosis of ectopic pregnancy was made where there was sonographic identification of an adnexal mass or gestational sac outside of the uterus[8]. If there was no evidence of either intrauterine or extrauterine pregnancy on the initial ultrasound scan, hCG level was taken into consideration. When hCG level was above the discriminatory zone (1500-2000 IU/L), ectopic pregnancy was diagnosed even when no adnexal mass was evident on ultrasound. Ectopic pregnancy was also suggested when there were abnormally rising (<53%) or plateauing hCG levels 48 hours apart and below the discriminatory zone[9]. In order to exclude the diagnosis of and administration of methotrexate to a failing pregnancy, even with the presence of an adnexal mass outside the uterus or hCG level above the discriminatory zone, two hCG levels 48 hours apart were required before administration of methotrexate.

Patients were not eligible for methotrexate treatment if they met the following criteria: haemodynamic instability; signs of peritonitis; abnormal baseline haematological, renal or hepatic laboratory values; and ectopic pregnancy with fetal cardiac activity.

Study Protocol

Patients received a single dose of methotrexate 50 mg/m^2, with the surface area calculated from a nomogram of height and body weight. The hCG level was measured on day 4 and day 7. The day of methotrexate administration was considered day 0. Optimal hCG drop was defined as a ≥15% decrease in hCG level between day 4 and day 7, followed by a weekly hCG level measurement until it became negative. When there was a <15% drop in hCG level between day 4 and day 7 following methotrexate administration, options including conservative management, second dose of methotrexate and surgery were offered to patients depending on symptoms, haemodynamic status, ultrasonographic findings, and post-methotrexate hCG levels. Treatment failure was defined as the need for additional methotrexate or surgical intervention.

Data including patients' demographic information, history of ectopic pregnancy, history of pelvic inflammatory disease, presenting signs and symptoms, hCG levels before and after treatment, and ultrasound results were recorded. In patients who were successfully treated with methotrexate the recovery time was also recorded, defined as the period from the day of methotrexate administration until the day of the last follow-up. Regular follow-up was provided until hCG level was <15 IU/L or a negative urine pregnancy test was obtained.

Statistical Analyses

To establish a cutoff value for hCG drop between day 4 and day 7 that would confirm treatment success, receiver operating characteristic (ROC) curve analysis using weighted Youden's Index was performed, defined as[10,11]:

Youden's Index: J = max {Sensitivity + r x Specificity - 1}
where *r* = (1 - prevalence) / (cost x prevalence).

The optimal cutoff was identified using cost as 1 and prevalence as the proportion of successful cases in the subject sample. The area under the curve, sensitivity, specificity, accuracy, as well as PPV and negative predictive value (NPV) at optimal cutoff were calculated.

Student's *t* test, Mann-Whitney *U* test, Pearson's

Chi-square test or Fisher's exact test was used where appropriate to compare the demographic and clinical characteristics of subjects with methotrexate treatment. Success rate of subjects with different hCG level was calculated. Pretreatment hCG level was stratified into four categories for comparison: <1000 IU/L, 1000-3999 IU/L, 4000-4999 IU/L, and ≥5000 IU/L. The failure rate of methotrexate has been reported to increase when initial hCG level is >4000 IU/L[12]. A systematic review reported an increase in failure when pretreatment hCG levels were >5000 IU/L[7]. Therefore, 4000 IU/L and 5000 IU/L were chosen as cutoff points. Variables with p<0.2 on univariate logistic regression were identified and further analysed by backward multivariate logistic regression. Student's *t* test was used to compare the recovery time between patients with an optimal drop in hCG level after methotrexate and those with a suboptimal drop.

All statistical analyses were performed using the Statistical Package for the Social Sciences Windows version 22 (SPSS Inc., Chicago [IL], US) and the R version 2.15.2 (R Foundation for Statistical Computing website: www.r-project.org). Statistical significance was set at p<0.05.

Results

A total of 1034 patients were diagnosed with ectopic pregnancy during the study period, and 182 received methotrexate. Six (3.3%) patients required a second dose of methotrexate and 32 (17.6%) required surgical intervention. The overall success rate was 79.1% (144/182).

Table 1 shows the demographic and clinical characteristics of the success and failure groups. There was no statistical significance between the two groups regarding patients' background including age, parity, history of

Table 1. Demographic and clinical characteristics of subjects with methotrexate treatment*

Characteristic	Success (n=144)	Failure (n=38)	Total (n=182)	p Value†
Age (years)	33 (30-37)	32.5 (28.8-36.3)	33 (29-37)	0.26
Parity				0.56‡
0	72 (50)	23 (61)	95 (52)	
1	57 (40)	14 (37)	71 (39)	
2	11 (8)	1 (3)	12 (7)	
3	4 (3)	0	4 (2)	
Ectopic pregnancy				0.73§
Yes	28 (19)	9 (24)	37 (20)	
No	116 (81)	29 (76)	145 (80)	
PID				0.75‡
Yes	12 (8)	4 (11)	16 (9)	
No	132 (92)	34 (89)	166 (91)	
Presence of adnexal mass in USG				0.78§
Yes	112 (78)	31 (82)	143 (79)	
No	32 (22)	7 (18)	39 (21)	
Maximum diameter of adnexal mass (mm)	19 (14-28)	20 (16-24)	19.0 (14.5-26)	0.99
Presence of free fluid in USG				0.38§
Yes	82 (57)	18 (47)	100 (55)	
No	62 (43)	20 (53)	82 (45)	
hCG level (IU/L)	1521.5 (624-2387.25)	2773 (1538.25-4365.75)	1621 (731.25-2970.75)	<0.001
hCG level drop (%)	32.86 (18.13-48.74)	1.38 (-4.57 to 13.41)	26.24 (10.85-44.07)	<0.001

Abbreviations: hCG = human chorionic gonadotrophin; PID = pelvic inflammatory disease; USG = ultrasonography

* Data are shown as No. (%) or median (interquartile range). Because of rounding, not all percentages total 100

† Mann-Whitney *U* test

‡ Fisher's exact test

§ Pearson's Chi-square test

ectopic pregnancy or history of pelvic inflammatory disease. There was no significant difference between the two groups for presence or absence of adnexal mass or free fluid in the pelvis on ultrasound. No significant difference was found between the two groups for size of adnexal mass. The pretreatment hCG level of the success group was significantly lower than that of the failure group (median: 1521.5 IU/L vs. 2773 IU/L; p<0.001). A statistically significant difference was also found between the two groups for the hCG drop between day 4 and day 7 after administration of methotrexate (median: 32.86% vs. 1.38%, p<0.001).

The ROC curve analysis using weighted Youden's Index was performed to determine the cutoff value to predict the success of a single dose of methotrexate (Figure). A total of 14 women were excluded because day-4 or day-7 hCG level was not available. Among 168 patients, 51 had hCG drop of <15% between day 4 and day 7 after methotrexate. The cutoff value was found to be 3.34% with a sensitivity of 94.29%, specificity of 57.14%, and accuracy of 88.10%. The PPV was 91.67% and the NPV was 66.67%. The positive likelihood ratio was 2.20 and the negative likelihood ratio was 0.10.

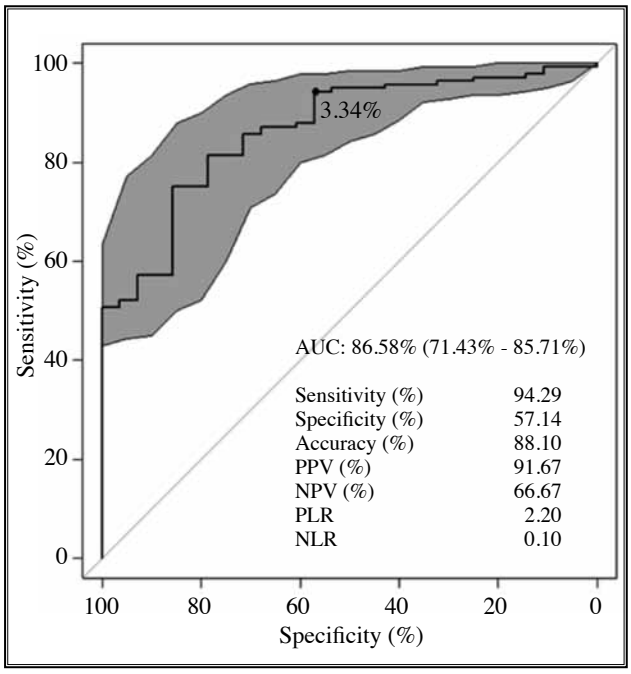

Figure. Receiver operating characteristic plot of the drop in human chorionic gonadotrophic level (%) after one dose of methotrexate

Abbreviations: AUC = area under the curve; NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV = positive predictive value

Pretreatment hCG levels were stratified into different categories for comparison (Table 2). The success rate reduced with an increase in hCG level. Multivariate logistic regression analysis revealed that pretreatment hCG level and drop in hCG level between day 4 and day 7 after methotrexate were factors that remained significant in prediction of success of methotrexate (Table 3). There was a significant reduction in the success rate for subjects with initial hCG value in the range of 1000-3999 IU/L (odds ratio [OR]=0.184, 95% confidence interval [CI], 0.046-0.739; p=0.02), 4000 to 4999 IU/L (OR=0.116, 95% CI, 0.017-0.786; p=0.03), and ≥5000 IU/L (OR=0.057, 95% CI, 0.008-0.417; p=0.01) when compared with those with initial hCG value of <1000 IU/L.

For patients who were successfully treated with one dose of methotrexate, the mean (±standard deviation) recovery time was 29.3 ± 11.8 days. The mean recovery time was 27.4 ± 11.0 days if hCG drop was ≥15% and 33.0 ± 10.9 days if hCG drop was between 3.34% and 15%. The difference between the recovery time of the two groups was significant (p=0.04).

Abdominal pain was the most common side-effect following methotrexate, present in 26 (18.1%) of all successful cases with six requiring hospital admission. Pain was nonetheless self-limiting and all patients were discharged 1 day after admission.

Outcome of the failed cases was also reviewed. There were 38 failed cases in total, of whom six required a second dose of methotrexate. Surgical intervention after methotrexate was required in 32 (17.6%) patients. Among them, 17 (53.1%) patients had a suboptimal drop or rising trend of hCG level and opted for surgery following counselling. Emergency admission was required by 15 (46.9%) patients. Mean day of presentation was 9.2 ± 6.9 days after methotrexate. Typical presentation was abdominal pain with peritoneal signs and ultrasound showed free fluid in the pelvis. No patient suffered haemodynamic instability and laparoscopic surgery was successful in all cases except one where conversion to laparotomy was required due to surgical difficulty associated with haemoperitoneum and dense pelvic adhesions. No failed case required admission to the intensive care unit. Among 22 patients with hCG drop of <3.34% between day 4 and day 7, 14 (63.6%) required surgical treatment or a second dose of methotrexate.

Discussion

The overall success rate of methotrexate in treating ectopic pregnancy in our study was 79.1%. Pretreatment

Table 2. Success rate of subjects with methotrexate treatment

hCG level (IU/L)	Total	Success	Success rate (95% confidence interval) [%]
<1000	62	58	93.55 (83.50-97.91)
1000-3999	96	73	76.04 (66.05-83.91)
4000-4999	11	6	54.55 (24.56-81.86)
≥5000	13	7	53.85 (26.12-79.60)
Overall	**182**	**144**	79.12 (72.35-84.63)

Abbreviation: hCG = human chorionic gonadotrophin

Table 3. Multivariate logistic regression analysis showing association between characteristics of subjects and the success of methotrexate treatment

	Adjusted odds ratio* (95% confidence interval)	p Value
hCG level (IU/L)		
<1000	1	NA
1000-3999	0.184 (0.046-0.739)	0.02
4000-4999	0.116 (0.017-0.786)	0.03
≥5000	0.057 (0.008-0.417)	0.01
hCG level drop (%)	1.070 (1.038-1.104)	<0.001

Abbreviations: hCG = human chorionic gonadotropin; NA = not applicable

* Adjusted odds ratio derived from backward multivariate logistic regression with variables including age, parity, hCG level, and hCG drop

hCG level and drop in hCG level between day 4 and day 7 were good predictors of success. When there was a ≥3.34% drop in hCG level between day 4 and day 7 after a single dose of methotrexate, the PPV for success was 91.67%. Comparing subjects with pretreatment hCG level of <1000 IU/L, there was a 8.61-fold decrease in the odds of treatment success for those with hCG level in the range of 4000 to 4999 IU/L and a 17.49-fold decrease when hCG level was ≥5000 IU/L.

Methotrexate inhibits DNA synthesis and cell proliferation. The time to achieve maximum concentration of methotrexate following intramuscular administration can vary from 30 to 60 minutes[13]. It combines with glutamate intracellularly to form methotrexate-polyglutamate (MTX-PG) by the process of glutamate polymerisation. The MTX-PGs are less able to be transported out of cells due to their large size, and serve as an intracellular storage pool of methotrexate. They can remain in the cells for a considerable period of time. This explains the efficacy of the single-dose methotrexate regimen for ectopic pregnancy. The disappearance of serum hCG after termination of pregnancy, whether intrauterine or extrauterine, follows a bi-exponential decay characterised by an initial rapid fall in hCG level during the first 48 hours with a half-life of 5 to 13 hours, followed by a slower phase with a half-life of 22 to 52 hours[14,15]. Before methotrexate comes into effect, the viable trophoblastic tissues produce hCG continuously. Therefore, the drop in hCG level following methotrexate can be variable.

Traditionally, a second dose of methotrexate should be administered if the hCG drop is <15% between day 4 and day 7 based on the single-dose methotrexate protocol[4]. No study has evaluated the outcome after a single dose of methotrexate when the drop between day 4 and day 7 is <15%. Our study found that the cutoff value to predict the success of a single dose of methotrexate was 3.34%. When there was a ≥3.34% drop in hCG level between day 4 and day 7 following administration of a single dose of methotrexate, 91.67% of patients had a successful outcome without need for a second dose or surgery. Therefore, if we can provide detailed pretreatment counselling and prompt access to medical care for all patients who receive a single dose of methotrexate, a more conservative approach with serial monitoring of hCG level can be considered when hCG drop is between 3.34% and 15%.

The mean recovery time in various studies has been reported to be 27 to 33 days after methotrexate[16,17]. All our

patients had regular follow-up until hCG level was <15 IU/L or a negative urine pregnancy test was obtained. The mean recovery time in our cohort was 29.3 ± 11.8 days. Women who had a ≥15% drop in hCG level following one dose of methotrexate had a statistically shorter mean recovery time than those who had a hCG drop between 3.34% and 15% (27.4 ± 11.0 days vs. 33.0 ± 10.9 days, p=0.04) . Both groups completed follow-up in a month.

The advantages of our study were that we had a homogeneous population, and all patients followed the same protocol for treatment and monitoring. There are some limitations to this study. It was a retrospective study in a Chinese population only with a small sample size, especially for the failure group. The decision to continue conservative management, proceed to a second dose of methotrexate or surgical intervention was not based on the drop in hCG level alone. The ultrasound findings, clinical condition, willingness to have regular follow-up visits and other factors that might affect patients' choices and these influences could not be assessed objectively. The study results might not be applicable to other ethnicities. We plan to design a prospective study to validate the new cutoff value of 3.34% for hCG drop between day 4 and day 7, and to confirm that it correctly identifies those who will achieve a successful outcome following a single dose of methotrexate.

Conclusions

Pretreatment hCG level and the hCG drop are good predictors of success of methotrexate in the management of ectopic pregnancy. When there was a ≥3.34% drop in hCG level between day 4 and day 7, the PPV for success without the need for a second dose of methotrexate was 91.67%. Therefore, a conservative approach with hCG monitoring can be considered when the hCG drop between day 4 to day 7 is ≥3.34%. A prospective study is required for validation.

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