ORIGINAL RESEARCH ARTICLE

Initial and Day Four β-hCG Levels as Predictors of Outcome of Single-dose Methotrexate Therapy in Medical Management of Tubal Ectopic Gestation

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Abstract

Introduction: Ectopic pregnancy is defined as the implantation of a fertilized ovum outside the endometrial cavity. The most common site is the fallopian tube. The incidence of ectopic pregnancy is 1–2% of all pregnancies. Methotrexate is the most commonly used drug for the medical management of ectopic pregnancy. It is a folic acid antagonist that prevents the growth of rapidly dividing cells including trophoblasts and fetal cells by interfering with DNA synthesis.

Aim and objective: To study initial and day 4 β-hCG levels as predictors of outcome of single-dose methotrexate therapy in the medical management of ectopic pregnancy.

Materials and methods: Thirty patients after confirmed diagnoses of tubal ectopic pregnancy were enrolled in the study. β-hCG was estimated using a sequential two-step immunoenzymatic (sandwich) assay.

Results: Out of 30 women who received inj MTX on day 0, 19 (63.3%) women were treated successfully with a single dose of methotrexate (group I), 8 (26.6%) women required a second dose of methotrexate, and 3 (10%) women required surgery. Women who required a second dose or surgery were treatment failure (group II) (36.6%) with a single-dose methotrexate regimen. The mean initial β-hCG β-hCG level was 2294.92 ± 1162.93 mIU/mL in group I and 3831.18 ± 1066.83 mIU/mL in group II. The difference of mean was statistically significant between group I and group II on day 0, day 4, and day 7.

Conclusion: Results in the present study favor therapeutic intervention with a second dose of methotrexate on day 4 against day 7 as in the current protocol in women with a rising trend of β-hCG between day 0 and day 4. However, due to the small sample size, further studies are needed to validate these findings.

Keywords: Drugs, Ectopic, β-hCG, Pregnancy.

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Introduction

Ectopic pregnancy is defined as the implantation of a fertilized ovum outside the endometrial cavity. The most common site is the fallopian tube. The incidence of ectopic pregnancy is 1–2% of all pregnancies. Common predisposing factors are prior tubal surgery, prior ectopic pregnancy, prior pelvic infection, prior contraceptive use, prior ovulation induction or artificial reproductive techniques for conception and cigarette smoking.[1] The diagnosis of ectopic pregnancy is complicated by a wide spectrum of clinical presentations from asymptomatic to acute. The early diagnosis of ectopic pregnancy is aided by a high index of suspicion in patients with risk factors. Because of the availability of simple diagnostic tools like high-resolution USG and sensitive β-hCG assay, early diagnosis and conservative management of ectopic gestation is possible.

Methotrexate is the most commonly used drug for the medical management of ectopic pregnancy. It is a folic acid antagonist that prevents the growth of rapidly dividing cells including trophoblasts and fetal cells by interfering with DNA synthesis.[2] Criteria for medical management include hemodynamic stability, serum β-hCG <5000 mIU/mL, gestational sac size <4 cm, absence of embryonic cardiac activity, and absence of free fluid in the cul-de-sac.[3] Management is done with a single- or multiple-dose methotrexate regimen. The single-dose methotrexate regimen includes the estimation of initial serum β-hCG level on day 0. If the patient fulfills the criterion of medical management 1st dose of Inj Methotrexate (50 mg/m^2) is given intramuscularly (IM) on day 0, followed by monitoring of β-hCG on day 4 and day 7. If β-hCG declines by 15% or more on day 7 in comparison to day 4, treatment is considered successful and β-hCG is monitored weekly until a value of <5 mIU/ML is obtained. If there is a rise or <15% fall in serum β-hCG between day 4 and day 7, then second dose of methotrexate is given IM on day 7, and β-hCG is monitored on day 11, 14, and weekly thereafter till a value of <5 mIU/ML is obtained. This is the most commonly used regimen for unruptured ectopic gestation with a success rate of 60–94%.[4]

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Hence, this study was planned to assess the efficacy of sustained elevation in serum β-hCG on day 4 when compared with day 0 in predicting unsuccessful response to single-dose methotrexate therapy in the management of tubal ectopic gestation and also to assess the efficacy of high initial β-hCG level in predicting unsuccessful response to single-dose methotrexate therapy in the management of tubal ectopic gestation.

Materials and Methods

The present study was conducted in the Department of Biochemistry, in collaboration with the Department of Obstetrics and Gynecology, Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi. Thirty women with unruptured tubal ectopic pregnancy who fulfilled the inclusion and exclusion criteria were recruited in the study.

Inclusion criteria stand similar to criteria for medical management of ectopic pregnancy while exclusion criteria include known case of methotrexate hypersensitivity, immunodeficiency, active peptic ulcer disease, any hepatic and renal dysfunction, and breastfeeding. After proper history and physical examination and confirmation of pregnancy by urine pregnancy test kit women with suspected tubal pregnancy were subjected to transvaginal and transabdominal USG to assess size and site of mass and fluid in the peritoneal cavity. After confirmation of ectopic pregnancy women who fulfilled the inclusion criteria were enrolled in the study after taking written informed consent.

Sample Collection

Venous blood (6 mL) was withdrawn from each subject using aseptic precautions in purple EDTA vacutainer for hematological investigation (analyzed same day) and 6 mL blood in red plain vacutainer for renal and liver function test and β-hCG estimation.

Estimation of β-hCG

Serum β-hCG was measured using Access Immunoassay Systems by Beckman Coulter Assess 2 (Chemiluminescent Assay).

Principle

The access total β-hCG assay is a sequential two-step immunoenzymatic (sandwich) assay. 10 µm L of serum sample was added to the reaction vessel with citrate buffer and incubated for 30 min. Afterward, a reagent containing rabbit anti-β-hCG alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-β-hCG complexes were added. Unbound materials were washed away and Lumi-Phos* 530 was added. Light generated was measured with a luminometer and β-hCG concentration was analyzed.

Statistical Analysis

IBM SPSS ver. 20 was used for various statistical analyses. Student's *t*-test was applied to the data confirming normal distribution. Categorical variables were compared using the Chi-square test between success and failure groups. Statistical analysis was expressed by mean + standard deviation. For all tests a probability <0.05 was considered significant. Charts and graphs were prepared using IBM SPSS ver. 20 and Microsoft Excel programs.

Results

In the present study, there was no statistically significant difference between group I (success) and group II (failure) in terms of age of women, obstetric status, gestational age at presentation, socioeconomic status, and presence of various predisposing factors for tubal ectopic presentation (Table 1).

Out of 30 women who received inj MTX on day 0, 19 (63.3%) women were treated successfully with a single dose of methotrexate, 8 (26.6%) women required a second dose of methotrexate, and 3 (10%) women required surgery. Women who required a second dose or surgery were treatment failure (36.6%) with a single-dose methotrexate regimen (Table 2).

The mean initial β-hCG β-hCG level was 2294.92 ± 1162.93 mIU/mL in group I and 3831.18 ± 1066.83 mIU/mL in group II. The difference of mean was statistically significant between group I and group II on day 0, day 4, and day 7 (Table 2 and Fig. 1). The mean day 4 β-hCG was significantly higher in group II compared with group I. Failure rate was 10%, 29%, 60%, and 63% when initial β-hCG was

Table 1: Outcome of medical management with single-dose methotrexate regimen

Outcome	*Number*	*%*
Success rate	19	63
Failure rate (2nd dose)	8	27
Failure rate (surgery)	3	10
Total	30	100

n = number of women

Table 2: Comparison of mean β-hCG values during treatment and therapeutic outcome

	Group I (Success)		*Group II (Failure)*	
Parameters	*Mean β-hCG (mIU/mL)*	*SD*	*Mean β-hCG (mIU/mL)*	*SD*
Day 0	2294.92	1162.93	3831.18	1066.83
Day 4	1773.89	895.70	4694.00	1438.64
Day 7	1091.26	439.80	5213.91	1659.78

p = <0.0001

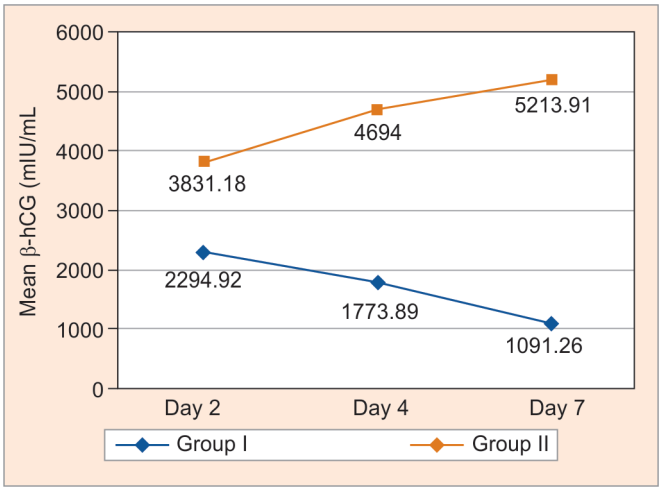

Fig. 1: Mean of β-hCG values during treatment and therapeutic outcome

between 1000–2000, 2001–3000, 3001–4000, and 4001–5000 mIU/mL respectively. The failure rate was increased (60–63%) at β-hCG levels above 3000 mIU/mL (Table 3). Women with a rising trend between day 0 and day 4 and high initial β-hCG (30,001–5000 mIU/mL) had 100% treatment failure. Women with a rising trend and lower initial β-hCG (1000–2000 and 2001–3000 mIU/mL) had a failure rate of 50% and 67%, respectively (Table 4). A rising trend on day 4 with a high initial β-hCG value of >3000 mIU/mL was associated with a 100% failure rate. Women with a declining trend between day 0 and day 4 with lower initial β-hCG (1000–3000 mIU/mL) had a 100% success rate and women with a declining trend with higher initial β-hCG 3001–5000 mIU/mL had a success rate of 60–67% (Table 5). Women with a declining trend between day 4 and day 7 with lower initial β-hCG 1000–2000 mIU/mL had a 100% success rate and women with a declining trend with higher initial β-hCG (3001–4000 mIU/mL and 4001–5000 mIU/mL) had a success rate of 67% and 75% respectively (Table 7). Six (20%) women had rising day 4 and rising day 7 β-hCG and all of them had treatment failure (required two doses). Four (13%) women had rising day 4 and falling day 7 β-hCG and two of them had treatment failure (underwent surgery) (Tables 8 and 9). Comparison of other variables between two groups is depicted in Table 10.

Table 3: Distribution of women according to outcome in various ranges of initial serum β-hCG

Initial β-hCG (mIU/mL)	*Total (n = 30)%*	*Group I (n = 19)%*	*Group II (n = 11)%*	*p value*
1000–2000	10 (33)	9 (90)	1 (10)	0.032
2001–3000	7 (23)	5 (71)	2 (29)	0.349
3001–4000	5 (17)	2 (40)	3 (60)	0.714
4001–5000	8 (27)	3 (39)	5 (63)	0.562
Total (*n*)	30 (100)	19 (63.3)	11 (36.6)	0.08

n = number of women

Table 4: Distribution of women with a rising trend of β-hCG β- between day 0 and day 4 in each initial β-hCG range according to the outcome

Initial β-hCG (mIU/mL)	*Rising day 4 β-hCG (n = 10)%*	*Group I (n = 2)%*	*Group II (n = 8)%*
1000–2000	2 (20)	1 (50)	1 (50)
2001–3000	3 (30)	1 (33)	2 (67)
3001–4000	2 (20)	0 (0)	2 (100)
4001–5000	3 (30)	0 (0)	3 (100)
Total (*n*)	10 (100)	2 (20)	8 (80)

N = number of women; *p* = 0.038

Table 5: Distribution of women with the declining trend of β-hCG between day 0 and day 4 in each initial β-hCG range according to the outcome

Initial β-hCG (mIU/mL)	*Declining trend of β-hCG (n = 20)%*	*Group I (n = 17)%*	*Group II (n = 3)%*
1000–2000	8 (40)	8 (100)	0 (40)
2001–3000	4 (20)	4 (100)	0 (40)
3001–4000	3 (15)	2 (67)	1 (33)
4001–5000	5 (25)	3 (60)	2 (40)
Total (*n*)	20 (100)	17 (85)	3 (15)

n = number of women; *p* = 0.047

Discussion

In the present study, initial β-hCG during medical management with inj MTX was significantly higher in group II compared with group I similar to study by Shaamash et al.,[5] Cohen et al.,[6] Elito et al.[7] and Eskander et al.[8] as given Table 6.

Table 6: Initial β-hCG during medical management with inj MTX was significantly higher

Study	*Year of study*	*Mean initial β-hCG in failure group (mIU/mL)*	*Mean initial β-hCG in success group (mIU/mL)*
Present study	2016	3831.18	2294.92
Shaamash et al.[5]	2015	3252	1873
Cohen et al.[6]	2014	2844	1601
Elito et al.[7]	1999	4828	1928
Eskander et al.[8]	2007	2701	1419

Table 7: Distribution of women with the declining trend of β-hCG between day 4 and day 7 in each initial β-hCG range according to the outcome

Initial β-hCG (mIU/mL)	*Total (n = 22)%*	*Group I (n = 19)%*	*Group II (n = 3)%*
1000–2000	9 (41)	9 (100)	0 (0)
2001–3000	6 (27)	5 (41)	1 (17)
3001–4000	3 (14)	2 (67)	1 (33)
4001–5000	4 (18)	3 (75)	1 (25)
Total (*n*)	22 (100)	19 (86.4)	3 (13.6)

n = number of women; *p* = 0.004

Table 8: Distribution of women with a rising trend of β-hCG between day 4 and day 7 in each initial β-hCG range according to the outcome

Initial β-hCG (mIU/mL)	*Total (n = 8)%*	*Group I (n = 0)%*	*Group II (n = 8)%*
1000–2000	1 (13)	0 (0)	1 (100)
2001–3000	1 (13)	0 (0)	1 (100)
3001–4000	2 (25)	0 (0)	2 (100)
4001–5000	4 (50)	0 (0)	4 (100)
Total (*n*)	8 (100)	0 (0)	8 (100)

n = number of women

Table 9: Relation between trends of β-hCG between day 0–4 and day 4–7 with therapeutic outcome

Trends of β-hCG on days 0–4 and days 0–7	*Total (n = 30)*	*Group I (n = 19)*	*Group II (n = 11)*
Rising D4 and rising D7 β-hCG	6 (20%)	0 (0%)	6 (100%)
Rising D4 and falling D7 β-hCG	4 (13%)	2 (50%)	2 (50%)
Falling D4 and rising D7 β-hCG	2 (7%)	0 (0%)	2 (100%)
Falling D4 and falling D7 β-hCG	18 (60%)	17 (94%)	1 (6%)

n = number of women

Table 10: Comparison of variables between two groups

Variables	*Group I (Success, n = 19)*	*Group II (Failure, n = 11)*
Treatment outcome	19 (63%)	11 (37%)
Mean initial β-hCG (mIU/mL)	2294.92 ± 1162.93	3831.18 ± 1066.83
Mean day 4 β-hCG (mIU/mL)	1773.89 ± 895.70	4694.0 ± 1438.64
Mean day 7 β-hCG (mIU/mL)	1091.26 ± 439.80	5213.91 ± 1659.78
Rising trend of β-hCG between days 0 and 4 (*n* = 10)	2 (20%)	8 (80%)
Falling trend of β-hCG between days 0 and 4 (*n* = 20)	17 (85%)	3 (15%)
Falling trend of β-hCG between days 4 and 7 (*n* = 22)	19 (86.4%)	3 (13.6%)
Rising D4 and rising D7 (*n* = 6)	0 (0%)	6 (100%)
Rising D4 and falling D7 (*n* = 4)	2 (50%)	2 (50%)
Falling D4 and rising D7 (*n* = 2)	0 (0%)	2 (100%)
Falling D4 and falling D7 (*n* = 18)	17 (94%)	1 (6%)

In our study, the mean day 4 β-hCG was significantly higher in group II compared with group I. Similar results were reported by Cohen et al.[6] and Mirbolouk et al.[9]

In the present study, the failure rate was 10%, 29%, 60%, and 63% when initial β-hCG was between 1000–2000, 2001–3000, 3001–4000, and 4001–5000 mIU/mL, respectively. The results showed that the failure rate was increased (60–63%) at β-hCG levels above 3000 mIU/ML. The result was similar to a study by Ustunyart et al.[10] In a study by Sagiv et al.,[11] failure rate was >50% at β-hCG levels above 4000 mIU/ML. In the present study, women with a rising trend between day 0 and day 4 and high initial β-hCG (30,001–5000 mIU/mL) had 100% treatment failure. Women with a rising trend and lower initial β-hCG (1000–2000 and 2001–3000 mIU/mL) had a failure rate of 50% and 67%, respectively. In a study by Skubisz et al.,[12] rising trend of β-hCG between day 0 and day 4 was associated with a 64% failure rate. A rising trend on day 4 with the high initial β-hCG value of >3000 mIU/mL was associated with a 100% failure rate. These findings could reliably be used to predict failure and initiation of 2 doses of MTX earlier on day 4 than the current standard protocol of day 7.

In the present study, women with a declining trend between day 0 and day 4 with lower initial β-hCG (1000–3000 mIU/mL) had a 100% success rate and women with a declining trend with higher initial β-hCG 3001–5000 mIU/mL had a success rate of 60–67%. Renukesh et al.[13] demonstrated that the declining trend of β-hCG between day 0 and day 4 was associated with a 75% success rate.

In the present study, women with a declining trend between day 4 and day 7 with lower initial β-hCG 1000–2000 mIU/mL had a 100% success rate and women with a declining trend with higher initial β-hCG (3001–4000 mIU/mL and 4001–5000 mIU/mL) had a success rate of 67% and 75%, respectively.

A combined high initial β-hCG of 3000 mIU/mL with a rising trend was associated with a 100% failure rate when compared with those who had only high initial β-hCG. Therefore, a high initial β-hCG of 3000 mIU/mL together with a rising trend of β-hCG between day 0 and day 4 is a strong predictor of treatment failure.

In the present study, six (20%) women had rising day 4 and rising day 7 β-hCG and all of them had treatment failure (required 2 doses). Four (13%) women had rising day 4 and falling day 7 β-hCG and 2 of them had treatment failure (underwent surgery). In the study by Wang et al.,[14] it was observed that for women who had a rising trend of β-hCG on days 0–4 and 4–7, 88.9% of them had treatment failure. For women who had a rising trend of days 0–4 and a falling trend of days 4–7, 57.1% of them had treatment failure. Falling day 4 and falling day 7 β-hCG was associated with a 2.2% failure rate. Results in the present study favor therapeutic intervention with a second dose of methotrexate on day 4 against day 7 as in the current protocol in women with a rising trend of β-hCG between day 0 and day 4. However, due to the small sample size, further studies are needed to validate these findings.

Conclusion

In the present study, there was no statistically significant difference between group I (success) and group II (failure) in terms of age of women, obstetric status, gestational age at presentation, socioeconomic status, and presence of various predisposing factors for tubal ectopic presentation. The mean initial β-hCG β-hCG level was 2294.92 ± 1162.93 mIU/mL in group I and 3831.18 ± 1066.83 mIU/mL in group II. The difference of mean was statistically significant between group I and group II on day 0, day 4, and day 7. A combined high initial β-hCG of 3000 mIU/mL with a rising trend was associated with a 100% failure rate when compared with those who had only high initial β-hCG. Therefore, a high initial β-hCG of 3000 mIU/mL together with a rising trend of β-hCG between day 0 and day 4 is a strong predictor of treatment failure. Results in the present study also favor therapeutic intervention with a second dose of methotrexate on day 4 against day 7 as in the current protocol in women with a rising trend of β-hCG between day 0 and day 4. However, due to the small sample size, further studies are needed to validate these findings.

Acknowledgments

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