Evaluation of Plasma Creatine Phosphokinase (CPK) Level Following a Single Injection of Methotrexate as a Predicator of Treatment Success in Ectopic Pregnancy

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Abstract

Objective: To evaluate the plasma creatine phosphokinase (CPK) level after a single injection of methotrexate (MTX) as a predictor of treatment success in ectopic pregnancy (EP).

Materials and methods: In this prospective study, seventy nine women older than 18 years treated with methotrexate for ectopic pregnancy were evaluated for CPK and β-subunit of human chorionic gonadotropin (βhCG) levels, while they received intramuscular MTX at a dose of 50 mg/m2. The day of injection was considered as day 1 (D1). CPK level on D1 was compared between the group 1(as treatment success group), treated by a single MTX injection, and the group 2, treated by two or three MTX injections or by surgery.

Results: The success rate of MTX treatment was 58 (73.3%). The mean of CPK was higher in treatment success group (group1) than failure group (group 2) (71.98 ± 15.711 vs. 64.43 ± 15.898), but the difference was not significant (p=0.06).The mean of βhCG was significantly lower in treatment success group (group 1) than failure group (group 2) (1187.52±631.45 vs. 1663.87±1096.845; p=0.01). Ultrasonographic findings of EP were seen in 63 patients, while the means of βHCG and CPK were higher in these patients than those with normal ultrasonography, but difference was not significant (p=0.37 and p=0.24, respectively).

Conclusion: The sample was not large enough to indicate a significant difference in the CPK level, which can be considered as an indicator for differentiating between the successful and unsuccessful treatment groups. Moreover, the present study did not show any relation between initial β-hCG and CPK serum levels, so our findings indicate that they are not possibly considered as two independent biomarkers in ectopic pregnancy.

Keywords: Methotrexate, CPK, Ectopic Pregnancy, Treatment

Introduction

Ectopic pregnancy (EP) is a life threatening condition with incidence of 2% in the general population (1). It is still the most important cause of first trimester maternal deaths, and is responsible for 73% of early pregnancy mortalities (2). Treatment of ectopic pregnancy (EP) with methotrexate (MTX) is an alternative to surgery (3, 4). Several studies have shown the use of methotrexate (MTX)is safe for treatment of early un ruptured EP in properly selected

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cases, while the risk of failure is the principal disadvantage, which then requires surgery (4,5).Methotrexate is an antagonist of folinic acid that inhibits dihydrofolic acid reductase interfering with DNA synthesis, repair, and cellular replication. Trophoblasts (rapidly proliferating tissues) are very sensitive to these MTX effects (6, 7). After a single dose (50 mg/m2) delivered by intramuscular injection (IM), the success rate of methotrexate treatment ranges from 64% to 94% (3). However, depending on patient selection criteria and different management, success rates may vary (5-8).

The first studies of MTX treatment for EP were conducted by Tanaka et al. (1982)(9)and Ory et al. (1986)(10). Prior studies indicated complete resolution of ectopic pregnancies in 94.2% of patients. Furthermore, they signified a very auspicious fertility rate among patients following this treatment, and indicated that 87.2% of them consequently had intrauterine pregnancies (11, 12).

The most commonly predictors of failure cited in these studies are serum human chorionic gonadotropin (β-hCG) progesterone levels, ectopic mass size, and the presence of ectopic cardiac activity (8, 13-15).Creatine phosphokinase (CPK) is an intracellular enzyme in muscle cells and its level increases in plasma in cases of muscle lysis, hence it is suitable for evaluation of the risk of tubal rupture in EP (16-18). A possible description is the presence of CPK inside trophoblastic cells. The elevated CPK would then be due to the trophoblastic invasion and the trophoblastic volume (16).The lysis of trophoblastic cells causes the plasma CPK level to increase; therefore, probability of a higher success rate for a single MTX dose is also greater (9-11,16-18).

Improvement of modalities of early diagnosis and treatment can reduce both mortality and morbidity of ectopic pregnancy. In this prospective study, we evaluated the plasma creatine phosphokinase (CPK) level after a single injection of methotrexate as a predictor of treatment success in ectopic pregnancy.

Materials and methods

We conducted this prospective study at Obstetrics and Gynecology Clinic of Shariati Hospital, Tehran, Iran , from 2011to 2012. Seventy nine women older than 18 years treated with MTX for EP were eligible for this study. The study protocol was approved by Committee of Ethics for Research in Obstetrics and Gynecology at Tehran University of Medical Sciences (TUMS). The study procedure was explained for all patients, and informed written consent was given before enrollment.

We enrolled patients referred to the center for pelvic pain and/or vaginal bleeding along with positive pregnancy test (EP suspicious). This diagnosis was confirmed with plasma β- hCG level higher than 2,000 IU/L without any visible intrauterine pregnancy documented by sonography test. In addition, the findings of sonography only revealed an inhomogeneous adnexal mass, an empty gestational sac with a hyperechoic ring. .

MTX therapy included the following criteria: absence of embryonic cardiac activity detected by transvaginal ultrasonography, β-hCG concentration<5,000 mIU/mL, ectopic pregnancy <4 cm in size as visualized by transvaginal ultrasonography, and the ability to participate in the follow-up.

Exclusion criteria were as follows: age under 18,sever systemic disease as acquired immune deficiency syndrome (AIDS), hepatitis and contraindications of MTX treatment in EP, like hepatic renal failure; thrombocytopenia; anemia; or any suspicion of tubal rupture (hemodynamic instability, severe pain, or detection of large hemoperitoneum by sonography).

The CPK and β-subunit of hCG (βhCG) level were measured in all included patients, then they received intramuscular MTX at a dose of 50 mg/m2. The day of injection was considered day 1 of the protocol (D1). Patients with suspected tubal rupture during treatment, or who refused second or third MTX injections were planned to undergo surgery. In addition, the need for surgery was determined by the surgeons blinded to the D1 plasma CPK level.

The measurements of βhCG titer were repeated on days 3 and 7 after the injection.

If the levels decreased by more than 15% between days 1 and 4 or between days 4 and 7, the patient were discharged, while the levels were subsequently measured at an ambulatory clinic. However, if βhCG titer decreased by less than 15%, (plateau levls), or rose between days 4 and 7, another MTX injection was offered. As stated earlier, hemodynamic instability, severe pain, and signs of peritoneal irritation were indications for immediate surgical intervention at any stage of the treatment protocol.

CPK levels on D1 were compared between group 1 (as treatment success group),treated by a single MTX injection, and group 2,treated by two or three MTX injections or by surgery.

The receiver operator characteristics curve was analyzed to assess the performance of the CPK level on D1 between groups 1 and 2.

Categorical variables were compared using the x^2 test, and continuous variables were compared using Student's t test. Correlation was tested by Spearman's rho. Differences were considered significant at $p<0.05$.

Results

Totally, we evaluated 79women with mean age of 28.7±3.42 and mean gestational age of 45.6±4.51 (Table 1). The mean of initial βhCG was 1314.14±802.943 and the mean of CPK was 69.97±11.993.The success rate of MTX treatment was 58 (73.3%).The correlation between βhCG and gestational age (p=0.035), and follow up duration and time to negative βhCG (p=0.001) were significant. The correlation among CPK with gestational age (p=0.11), βhCG(p=0.59) and time to negative βhCG (p=0.12) were not significant.CPK and βhCG revealed no significant correlation with age, gravity, parity, follow up duration, EP history, abortion history and infertility history (Tables 1&2).Moreover, the serum level of βhCG and CPK did not show significant difference between normal and infertile patients(P=0.86 and P=0.49 respectively) (Table 2).

The levels of βhCG and CPK did not correlate significantly with clinical characteristics; however, the correlation between βhCG with spotting was significant (p=0.021). Our results confirmed that 63 patients showed ultrasonographic signs of EP, while the mean levels of βhCG and CPK were higher in these patients than patients with normal ultrasonography, but difference was not significant (p=0.37 and p=0.24, respectively)(Table 3).The mean of βhCG level was significantly lower in group1than group 2 (1187.52±631.45 vs.16663.87±1096.845; p=0.01).The mean of CPK level was higher in group1than group2 (71.98±15.711 vs.64.43±15.898), but the difference was not significant(p=0.06)(Table4).

Table 1: Demographic characteristics of patients

Age (mean± SD)	28.7± 4.31
Gestational age (mean± SD)	45.67± 7.31
Follow up (Week) (mean± SD)	2.43± 0.77
Gravid1	36 (45.6%)
Gravid 2 or more	43 (54.4%)
abortion	15 (19%)
infertility	15 (19%)
EP history	3 (3.8%)
Pregnancy	67 (84.4%)
IVF	5 (6.3%)
IUI	1 (1.3%)
Clomiphene ,HMG	6 (7.6%)
spotting	38 (48.1%)
Abdominal pain	31 (39.2%)
Group 1(single dose MTX)	58 (73.4%)
Group 2(repeated dose or surgery)	21 (26.6%)
Laparoscopy	9 (11.4%)
Laparotomy	7 (8.9%)
Second MTX dose	5 (6.4%)
EP diagnosed in ultrasonography	63 (79.7%)

Table2: Correlation between βhCG and CPK levels between normal and infertile patients

Marker	History of infertility	n	Mean± SD	P
βhCG	no	64	1306.66± 841.074	0.86
	yes	15	1346.07± 638.221	
CPK	no	64	70.58± 16.841	0.49
	yes	15	67.40± 11.993	

Table3: Themean ± SDof βhCG and CPK levels in normal and EP patients

Marker	Ultrasonography	n	Mean± SD	P
βhCG	Normal	16	1153.13± 589.746	0.37
	EP	63	1355.04± 847.680	
CPK	Normal	16	65.75± 13.334	0.24
	EP	63	71.05± 16.548	

Table 4: The means of βhCG and CPK levels in two groups

Marker	Group	n	Mean± SD	P
βhCG	success	58	1187.52± 631.458	0.01
	failure	21	1663.87± 1096.845	
CPK	success	58	71.98± 15.711	0.06
	failure	21	64.43± 15.898	

Discussion

In our experience, the success rate of MTX treatment was 58 (73.3%), which is lower than recent studies reporting success rates of 88 to 91 % (3,5,19). An explanation for such discrepancy between our experience and previous reports may due to different criteria in selection of patients, study design, and different definition of success. In current study, only patients responding to a single MTX injection were considered as treatment success group (group 1), but some studies considered patients with two or more injections as treatment success group (3,5). In current experience, when the CPK level on first day exceeded109 IU/L, the sensitivity and specificity of group 1 were about 35.3% and 100%, respectively. It explains that the positive predictive rate was 100%, which it means that 100% of patients required surgical treatment or several MTX injections had a CPK level on first day <109 IU/L, while 100% of those with a CPK level>109 IU/L were successfully treated with a single MTX injection (19).

In line with this hypothesis, the mean of CPK was higher in group 1 than group 2 (71.98 ± 15.711 vs. 64.43 ± 15.898), but the difference was not significant (p=0.06). However, Gnisci et al. (2011) reported significant higher CPK plasma level in EP patients treated successfully compared to the control group (98±44 vs.69±18; p=0.004).They concluded that creatine phosphokinase level predicts outcome of women who were successfully treated for ectopic pregnancy with only a single injection of methotrexate(19).

Several studies indicated significant higher level of CPK in ectopic pregnancy (20-22). In a comparative study, Asgharnia et al. (2012) evaluated the role of serum creatine phosphokinase in diagnosis of tubal ectopic pregnancy. They enrolled 111 patients into three groups, and indicated that the mean CPK in group 1(EP) was significantly more than group 2(threatened abortion) and group 3(normal pregnancy) [96.27±63.9 u/lit (group 1) vs. 55.37±14.1 u/lit (group 2) and 48.94±19.2 u/lit (group 3); p=0.001].They deduced that determination of total CPK can enhance the diagnostic value of tubal pregnancy(20).In another case-control study by Saha et al. (1999), 20 women with EP and 20 women with normal pregnancy were studied. Total CPK level was significantly higher in EP group (34.15±1.17 U/L) compared to the control group (18.72±1.25)(23).Additionally, Lavie et al. (1993) studied three groups of 17 patients classified for EP, abortion and normal pregnancy. In their experience, the CPK level was >45 U/Lit for all patients with tubal pregnancy, that was significantly higher than those in abortion and normal pregnancy groups (22).

However, previous experiences have prepared conflicting information about diagnostic value of CPK for EP, some authors refused these results and signified that CPK cannot be useful diagnostic marker for EP(23).A possible explanation for such discrepancy can be explained by different gestational ages, different location of EP and the degree of tubal distention; furthermore, the serum biomarkers often do not follow a steady pattern over a normal gestation and techniques used to detect the different biomarkers (24). Several studies indicated that the βhCG is considered as a more useful biomarker in EP detection(23-26).In current survey, the mean of initial βhCG was lower in treatment success group than failure group (1187.52±631.45 vs.1663.87±1096.845; p=0.01). In line with our results, Nowak-Markwitz et al. (2009) reported the medians of βhCG levels after the first dose of MTX was significantly lower in successful than failure groups (564 vs. 4049 mIU/mL); furthermore, they indicated when the βhCG level is >1790 mIU/mL, the MTX treatment of ectopic pregnancy is at risk of failure(25).Moreover, Colins et al. (2008) in a systematic review showed an increase in failure treatment with single-dose methotrexate when the initial hCG is above 5,000 mIU/mL(26).

Relatively small sample size and absence of control group was two limitations of our experience .When the sample was not large enough, the obtained result could not indicate a significant difference in the CPK level, which seems to be the best indicator differentiating between the successful and unsuccessful treatment groups. Moreover, the present practice did not show any relation between initial hCG and CPK serum levels.(, a finding indicating that they seem to be two independent biomarkers)We recommend more controlled studies with larger series in order to validate results reported here.

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