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ORIGINAL ARTICLE

Evaluation of Ovarian Reserves of Patients who Have Received Methotrexate Treatment Due to Ectopic Pregnancy

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Abstract

Introduction: The study aimed to investigate the effects of treatment on ovarian reserve in patients who received a single dose of methotrexate (MTX) due to ectopic pregnancy.

Methods: This prospective study included patients who were diagnosed with ectopic pregnancy in the gynecology and obstetrics clinic of our hospital and met the criteria. The sociodemographic and clinical data of the patients were recorded. Then, MTX was intramuscularly administered at a dose of 50 mg/m2 to eligible patients. Single-dose MTX treatment was considered sufficient in patients with a 15% decrease in serum beta-human chorionic gonadotropin (ß-HCG) concentration between the 4th and 7th days. Weekly ß-HCG follow-ups were undertaken until the ß-HCG levels were negative (5 U/mL). Before starting treatment, serum ß-HCG, complete blood count, and kidney and liver function tests were conducted in all patients. Follicle-stimulating hormone (FSH) and anti-Mullerian hormone (AMH) levels were investigated to evaluate ovarian reserves. 6 months after treatment, the ovarian reserve tests were repeated. The values recorded before and 6 months after treatment were statistically compared.

Results: Thirty-four patients who were diagnosed with an ectopic pregnancy over the study period and met the specified criteria were evaluated. The mean values of investigated parameters were years for age, gravida, parity, body mass index, cm for ectopic focus size, and the baseline β-HCG. Before MTX treatment, the AMH and FSH values were reported. At the end of 6 months after MTX, the AMH and FSH values were determined, respectively. There was no significant difference in the statistical comparison of the serum AMH values before and after 6 months of treatment (p>0.488).

Discussion and Conclusion: Single-dose MTX administration for ectopic pregnancy treatment had no significant effect on ovarian reserves measured according to the parameters mentioned above.

Keywords: Anti-mullerian hormone; ectopic pregnancy; methotrexate; ovarian reserve.

Ectopic pregnancy is defined as the implantation of the fertilized ovum anywhere outside the uterus. The incidence of ectopic pregnancy is approximately 1–2% in all pregnancies. It is the leading cause of pregnancy-related deaths in the first trimester and is responsible for up to 6% of maternal deaths during early pregnancy[1,2]. Today, the frequency of ectopic pregnancy is increasing. Among the main reasons are the more frequent occurrence of pelvic infections, increased use of intrauterine devices, and widespread use of assisted reproductive techniques. Obser-

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vation of ectopic pregnancies also in completely normal tubes suggests that impairment of pregnancy material or maternal hormonal differences play a role in the etiology[3]. Other important reasons for the increase in the reported frequency of ectopic pregnancy include increased possibilities of early and definitive diagnosis with the growing use of reconstructive tubal surgical interventions and precise human chorionic gonadotropin (HCG) measurements, transvaginal ultrasonography (USG), and laparoscopy. In the diagnosis of ectopic pregnancy, anamnesis, physical and gynecological examination, USG, serum beta-HCG (ß-HCG) levels, culdocentesis, dilatation curettage, and laparoscopy are used. Ectopic pregnancy treatment can be performed medically or surgically or patients can be followed up without treatment, depending on various factors such as clinical condition, location and week of ectopic pregnancy, serum ß-HCG levels, and presence of fetal cardiac activity. In patients with ectopic pregnancy presenting with low ß-HCG levels, available options are observational management, medical treatment with methotrexate (MTX), or surgical methods, including salpingectomy, salpingostomy, tubal milking, and fimbriectomy. A patient with diagnosed ectopic pregnancy should be immediately transferred for surgery if she has hemodynamic instability, the initial ß-HCG level is high, fetal cardiac activity is detected outside of the uterus on USG, or there is a contraindication to medical management[4]. MTX is the most commonly used method as an alternative to surgical treatment, and it is suitable for selected patient groups. It is an antimetabolite drug that acts on actively growing cells. This mechanism effectively treats EP; however, it is supposed that MTX has an impact on fertility by targeting the actively dividing granulosa cells (GC) within the ovary. MTX is a folic acid antagonist, an antimetabolite drug that acts on actively growing cells, causes disruption of protein synthesis, and exerts a cytotoxic effect on cells in the synthesis phase. This mechanism effectively treats EP; however, it is supposed that MTX has an impact on fertility by targeting the actively dividing GC within the ovary. There is no perfect test that fully shows ovarian reserve. It is preferred that the test showing ovarian reserve gives results independently of the cycle. Several tests are used in daily practice to have an idea about ovarian reserve (anti-Mullerian hormone [AMH], inhibin B, estradiol, follicle-stimulating hormone [FSH], and luteinizing hormone [LH]). AMH is an endocrine marker considered to evaluate ovarian reserve[5]. The AMH is secreted from GC, not affected by gonadotropins, and at any time of the menstrual cycle. This makes it a unique and specific test for evaluating ovarian reserve[6]. Therefore, the value of MTX administration in women with ectopic pregnancy is unequivocal[7].

Medical treatment to be applied in ectopic pregnancy has many benefits, including less tubal damage, low cost, and although controversial, higher potential of future fertility. The current study aimed to prospectively evaluate 34 cases diagnosed with tubal ectopic pregnancy in our clinic. The effect of MTX treatment applied to the patients on their ovarian reserves was statistically evaluated. We hypothesized that single-dose MTX treatment does not affect ovarian reserve in ectopic pregnancies.

Materials and Methods

This prospective clinical study was carried out between January 2012 and December 2015 at the gynecology and obstetrics clinic of Adana Numune Training and Research Hospital affiliated with the Turkish Ministry of Health after obtaining approval from the Ethics Committee of the hospital (Date: October 02, 2015, Number: 200). The study was designed in accordance with the principles of the Declaration of Helsinki. Thirty-four female patients aged 17–41 years, who were diagnosed with ectopic pregnancy in the obstetrics and gynecology clinic of our hospital, were included in the study. Patients with endometriosis, premature ovarian insufficiency, previous ovarian surgery, or oophorectomy, those who received a second dose of MTX due to the failure of a single dose, and those with impaired hemodynamics during the treatment process were excluded from the evaluation. All patients' age, height, weight, gravida, parity, abortion, history of operation, smoking, history of pelvic inflammatory disease, and additional diseases were recorded. Ectopic pregnancy was diagnosed based on the patient's anamnesis, gynecological examination, physical examination, pregnancy test, USG, and dilatation curettage data. The laboratory findings of serum β-HCG, complete blood count, kidney and liver function tests, FSH, and AMH were also obtained for all patients before starting treatment. All patients were informed about ectopic pregnancy, explaining possible risks. Patients with no signs or symptoms of rupture, hemodynamic stability, normal kidney and liver function tests, an ectopic focal size smaller than 4 cm, βHCG value <10,000 IU/mL, and no fetal cardiac activity were given information about the treatment protocol, and the single-dose MTX treatment was started. MTX was intramuscularly administered at a dose of 50 mg/m^2 to the eligible patients. Serum ß-HCG values were measured on the 4th day of treatment. Increases in serum ß-HCG concentration from day 1 to day 4 were considered normal. Single-dose MTX treatment was

considered sufficient in patients with a 15% decrease in serum ß-HCG concentration from day 4 to day 7. Weekly ß-HCG follow-ups were performed until ß-HCG levels became undetectable. Since AMH is one of the most important parameters showing ovarian reserves regardless of the menstrual cycle, the AMH test was undertaken before and after treatment. 6 months after the treatment, the FSH and AMH tests were repeated. The baseline and post-treatment 6th-month values were statistically compared. The power of the research was calculated with the G*power program. As a result of the analysis made with the AMH differences detected between the addicted groups, the power of the research was calculated as 71%, and as a result of the analysis made with the FSH differences found, it was calculated as 99%.

Statistical Analysis

Data analysis was performed using the statistical package for the social sciences (Inc, Chicago, Illinois, USA) v. 16.0. Numerical data were collected as continuous and discrete values. The mean and median values were used as the central distribution criteria for the data. Standard deviation and minimum and maximum values were used as the prevalence criteria. If the assumptions were met, the paired samples t-test was used as a parametric test. $p<0.05$ was accepted as statistically significant.

Results

Thirty-four patients who were diagnosed with an ectopic pregnancy over the study period and met the specified criteria were evaluated. Six patients with a history of infertility, three with a history of endometriosis, and 10 who did not respond to MTX treatment and underwent surgery were excluded from the evaluation. The presenting symptoms and physical examination findings of the patients, smokers or not smokers, are given in Tables 1 and 2, respectively. According to anamnesis, had no history of abdominal operation, had undergone an appendectomy, tubal-ovarian surgery, or other abdominal surgeries, and a previous history of inpatient or outpatient treatment with a diagnosis of pelvic inflammatory infection were present as shown in Table 1. The mean AMH and FSH values before MTX treatment and at the end of 6 months after MTX treatment were measured, respectively, as shown in Table 3. There was no statistically significant difference in the comparison of AMH before and at 6 months after treatment ($p>0.488$). However, a statistically significant difference was observed in the comparison of the FSH values ($p<0.000$), which was attributed to the suppressor effect of pregnancy.

Table 1. Signs and general characteristics of the patients

	n=34	Percentage
Abdominopelvic pain	77.4	
Vaginal bleeding	45.2	
Bleeding and pain	35.5	
Nausea and vomiting	12.9	
Abdominal tenderness	80.6	
Vaginal bleeding	48.4	
Sensitivity to collum stretching	45.2	
Fullness in the pouch of Douglas	41.9	
History of PID	Absent 24	70.6
	Present 10	29.4
History of abdominal surgery	Appendectomy 1 (34)	2.9
	None 27 (34)	79.4
Tubo-ovarian surgery	4(34)	11.8
Other surgery	2(34)	5.9

PID: Pelvic inflammatory disease.

Table 2. Demographic characteristics of the patients

Age	28.29±5.33
Gravida	2.88±1.49
Parity	1.03±0.90
Body mass index	27.08±6.14
Focus size (cm)	2.32±0.82
Baseline β-HCG (mUI/mL)	1.581.05±1.656.41
Smoking status	
Smoker	7 (20.6%)
Non-smoker	27 (79.4%)

β-HCG: Beta-human chorionic gonadotropin.

Table 3. Comparison of the AMH and FSH values before and after MTX treatment

	n=34	p
Baseline AMH (ng/mL)	3.54±2.08	0.488
6th-month AMH (ng/mL)	3.70±2.15	
Baseline FSH (mlU/mL)	2.55±2.55	0.001
6th-month FSH (mlU/mL)	5.76±2.12	

FSH: Follicle-stimulating hormone; AMH: Anti-Mullerian hormone; MTX: Methotrexate.

Conclusion

This study evaluated the effect of MTX on ovarian reserves in patients who had received a single dose of MTX treatment due to ectopic pregnancy. It was aimed to compare ovarian reserves before and after treatment. Patients who

received only one dose of MTX and were followed up until their β-HCG values became negative were included in this evaluation. When the studies examining the change of AMH during pregnancy are evaluated as a whole, it has been determined that AMH decreased until the 36th week following the increase in the first trimester, and after birth, it reached a plateau slightly above the pre-pregnancy values, but this increase was not statistically significant[8]. Considering the biochemical kinetics of AMH, we got the control value 6 months later. As it is known, a significant number of hormonal changes occur during the pregnancy period. With negative feedback from increased serum estradiol, progesterone, and inhibin levels during pregnancy, the FSH and LH levels decrease to an almost undetectable level in the term; however, in the early weeks of pregnancy, negative hormonal feedback is not as much as in term. Since there is already an ectopic pregnancy, it would not be right to expect hormonal suppression as much as in a normal healthy intrauterine pregnancy. It is the most widely used FSH and LH measurement to learn ovarian reserve. AMH is the most popular biomarker of ovarian reserve, as it provides a direct determination of ovarian status[9]. In recent studies, as one of the most important parameters showing ovarian reserve independent of the menstrual cycle, AMH has been used to evaluate ovarian reserves in patients before and after treatment. MTX is the most commonly used pharmacological agent in the medical treatment of ectopic pregnancy[10]. MTX is a folic acid antagonist and functions by inhibiting the dihydrofolate reductase enzyme, which is involved in DNA repair and cell division and inhibits the conversion of dihydrofolate to tetrahydrofolate[11]. Although MTX affects cell division in the whole phase, it affects cells, especially in the S-phase. In addition, rapidly dividing bone marrow, gastrointestinal cells, and primordial cells in the ovaries are more affected by MTX treatment. Many researchers have considered that MTX negatively affects oocyte production; however, this effect is reversible[12]. It is already known that primordial follicles, which are in their growth stages, are more resistant to ischemia than the follicle population, due to the low metabolic rate at rest. If the number of primordial follicles remaining in the resting pool is sufficient, it can help regenerate the growing follicles and replace damaged growing follicles upon cessation of treatment[13]. Although the effect of chemotherapeutic agents on the ovaries is not yet precisely known, it is predicted that they induce apoptosis on ovarian follicles and reduce ovarian reserve[14]. If the number of primordial follicles in the resting state in the ovaries is sufficient, discontinuation of MTX treatment helps regenerate growing follicles. Primordial follicles are likely to replace damaged growing follicles in ectopic pregnancy. This effect varies according to the dose and duration of action of the agent used. Some studies have suggested that AMH, basal antral follicle count (AFC), FSH, E2, and ovarian volume can be used in the evaluation of ovarian reserve after a single dose of MTX. In a study conducted by Oriol et al.,[15] in patients who received a systemic single dose of MTX treatment due to ectopic pregnancy, ovarian reserves were evaluated based on AMH and shown not to be affected by treatment. In a study examining whether MTX treatment used for ectopic pregnancy affected the future fertility of women with a history of undergoing assisted reproductive technology, Boots et al.[16] did not find a significant difference in FSH, AFC, stimulation times, oocytes removed, or fertilization rate. At the end of the study, they showed that MTX does not influence ovarian reserve, and MTX remains a safe-effective treatment option for woman who is suitable for medical treatment. McLaren et al.[17] reported a statistically significant decrease in oocyte yield within 6 months following MTX treatment; however, in vitro fertilization cycles examined after 6 months did not show a decrease in oocyte yield. In another study, Sahin et al.[18] determined that in the treatment of ectopic pregnancy, neither single-dose MTX application nor salpingectomy had a permanent harmful effect on the ovarian reserve, with the serum AMH and AFC levels remaining unchanged in the long term. Shirazi et al.[19] assessment of single-dose MTX treatment on ovarian reserve in a woman with ectopic pregnancy. İn their study, AMH, AFC, FSH, and LH were evaluated before and 8 weeks after treatment although the time is short AMH did not significantly vary after the administration of MTX compared to before and after value.

In conclusion, our study showed that ovarian reserves were not affected in the early period of patients who received a single dose of MTX treatment due to ectopic pregnancy. However, further studies are needed to demonstrate the possible effects over a longer term.

Ethics Committee Approval: This prospective clinical study was carried out between January 2012 and December 2015 at the gynecology and obstetrics clinic of Adana Numune Training and Research Hospital affiliated with the Turkish Ministry of Health after obtaining approval from the Ethics Committee of the hospital (Date: October 02, 2015, Number: 200).

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