Effect of Methotrexate on Anti-Mullerian Hormone Levels, β-hCG and Tumor Size in Women with Low-Risk Gestational Trophoblast Disease

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Abstract

Introduction: This study aimed to evaluate the effect of methotrexate (MTX) chemotherapy on anti-mullerian hormone (AMH) levels, human chorionic gonadotropin (HCG) levels, and tumor size in women with gestational trophoblastic disease (GTD).

Method: This study was conducted at Hasan Sadikin General Hospital, Bandung, West Java, from April to October 2020. The AMH level, beta human chorionic gonadotropin (ß-hCG) and tumor size in women with a low risk of GTD prior to and after MTX chemotherapy treatment were measured and compared.

Results: Our study found a reduction in mean AMH level to 0.82 ng/ml after the MTX chemotherapy. The mean AMH level after chemotherapy in women with low-risk GTD decreased to 0.82 ng / ml. In addition, ß-hCG level decreased after chemotherapy with MTX. There was a negative relationship between ß-hCG level and tumor size before and after chemotherapy. Higher ß-hCG levels and tumor size before chemotherapy resulted in a further increase in AMH after chemotherapy.

Discussion: There was a decrease in AMH and ß-hCG levels after three cycles of MTX chemotherapy in women with low-risk GTD. Tumor size and ß-hCG correlated with post-chemotherapy AMH results.

Key words: Chemotherapy, Methotrexate, Anti-mullerian hormone, ß-hCG, Tumor gestational trophoblast

Pengaruh Metotreksat terhadap Kadar Hormon Anti Mullerian, β-hCG dan Ukuran Tumor pada Wanita dengan Penyakit Trofoblas Gestasional Risiko Rendah

Abstrak

Pendahuluan: Penelitian ini mengevaluasi efek kemoterapi metotreksat (MTX) terhadap kadar hormon anti-mullerian (AMH), kadar human chorionic gonadotropin (HCG), dan ukuran tumor pada wanita yang didiagnosis penyakit trofoblas gestasional (GTD) risiko rendah.

Metode: Penelitian ini dilakukan di Rumah Sakit Umum Pusat Hasan Sadikin, Bandung, Jawa Barat, dari bulan April hingga Oktober 2020. Kadar AMH, beta human chorionic gonadotropin (ß-hCG), dan ukuran tumor pada wanita dengan GTD risiko rendah sebelum dan sesudah pengobatan kemoterapi MTX diukur dan dibandingkan.

Hasil: Pada penelitian kami menemukan penurunan kadar AMH rata-rata menjadi 0,82 ng/ml setelah kemoterapi MTX. Rata-rata kadar AMH setelah kemoterapi pada wanita dengan GTD risiko rendah menurun menjadi 0,82 ng/ml. Selain itu, kadar ß-hCG juga menurun setelah kemoterapi dengan MTX. Terdapat hubungan negatif antara kadar ß-hCG dan ukuran tumor sebelum kemoterapi dan AMH setelah kemoterapi. Kadar ß-hCG yang lebih tinggi dan ukuran tumor sebelum kemoterapi menunjukkan peningkatan lebih tinggi pada AMH setelah kemoterapi.

Kesimpulan: Terjadi penurunan kadar AMH dan ß-hCG setelah tiga siklus kemoterapi MTX pada wanita dengan GTD risiko rendah. Ukuran tumor dan kadar ß-hCG berkorelasi dengan hasil kadar AMH setelah kemoterapi.

Kata kunci: Kemoterapi, Metotreksat, Hormon anti-mullerian, ß-hCG, Tumor trofoblas gestasional

Introduction

Cancer is one of the most common cause of death worldwide. According to data from The International Agency for Research on Cancer in 2018, the incidence rate of cancer and its mortality rate have steadily increased up to 18.1 million new cases and 9.6 million deaths.[1] Additionally, the total five-year survival prevalence in the world is estimated to be around 43.8 million.[2] Indonesia has a relatively high prevalence of malignancies, accounting for 1.4 per 100 inhabitants or about 347,792 people.[3]

Gestational trophoblastic disease (GTD) is one of the malignancies in women of reductive age that responded well to chemotherapy agents and is reported to have a good prognosis.[4] Gestational trophoblastic tumor occurs in 1:40,000 pregnancies in Europe and North America, whereas in Asia it is reported to be more than nine times higher with the rate of 9.2:40,000 pregnancies.[5]

In Hasan Sadikin General Hospital, there were 166 patients with GTD and approximately 80% of the patients received chemotherapy.[6,7,8] The chemotherapy protocol used for low-risk GTD in Hasan Sadikin General Hospital is methotrexate (MTX) with a dose of 1 mg/kg body weight given intramuscularly (IM) four times on the first, third, fifth and seventh day and 15 mg folinic acid IM in between, with additional consolidated therapy for 2 – 3 cycles.[4,9,10]

The current reports on decreased ovarian serve as MTX side effects still vary. Two studies conducted in China and Japan observed the decreased ovarian reserve in GTD patients after MTX chemotherapy.[11,12] MTX administration will compete with folic acid in entering granular cell membranes through active transport, resulting in decreased ovarian reserve. Furthermore, MTX decreases the lower ovarian reserve through both direct and indirect effects. The direct effect occurs within 24 h post-administration where MTX inhibits the dihydrofolate reductase enzyme that is necessary for the synthesis of thymidylate and purines which are integral parts of DNA and RNA synthesis, respectively. On the other hand, the indirect effects of MTX are mediated by its conversion into a series of polyglutamate-MTX (MTX-PG), which has a good affinity with tumor and chorionic cells. Both direct and indirect effects decrease follicle reserves in the ovaries.[13,14] However, to the best of our knowledge, there is still no research on the impact of MTX chemotherapy on the Indonesian population with GTD.

The ovarian reserve can be assessed using several markers, including the AMH levels. Research analyzing the impact of MTX on AMH levell reduction in GTD patients is an intriguing area of study. Additionally, the International Federation of Gynecology and Obstetrics highly recommends the evaluation of human chorionic gonadotropin (ß-hCG) levels and tumor size before and after MTX chemotherapy. Ovarian reserve is a necessary measure to observe the success of fertility maintenance after chemotherapy in GTD patients.[15] Therefore, this study aimed to assess the impact of chemotherapy on ovarian reserve, using AMH levels, human chorionic gonadotropin (ß-hCG) levels, and tumor size following MTX chemotherapy to predict patient fertility and evaluate the side effects of chemotherapy in GTD patients of reproductive age.

Method

The design of the study is a prospective hospital-based study, 54 women aged 20-40 years old with low-risk GTD candidates for MTX chemotherapy at Dr. Hasan Sadikin Hospital, Bandung Indonesia were included. The study was conducted between April and October 2020. The exclusion criteria were history of ovarian surgery, thyroid dysfunction, hyperprolactinemia, Cushing

syndrome, polycystic ovarian syndrome, and withdrawal from continuing chemotherapy.

History taking was performed to assess any history of PCOS, thyroid dysfunction, hyperprolactinemia, or Cushing syndrome. Standard physical and laboratory examinations were also performed in accordance with the therapy guidelines of the Obstetrics and Gynaecology Department, Hasan Sadikin General Hospital Bandung. Women with a low-risk of GTN were diagnosed on the basis of their history, physical examination, anatomical pathology examination, ultrasound, complete blood laboratory, quantitative ß-hCG levels, and radiology. There was no special preparation before blood sampling.

The study measured AMH levels in low-risk GTD patients before and after MTX chemotherapy and analyzed the correlation between ß-hCG levels and tumor size before chemotherapy with post-MTX chemotherapy AMH levels.

MTX chemotherapy was administered with a dose of 1 mg/kg body weight given IM four times, on the first, third, fifth, and seventh day with additional 15 mg folinic acid IM on the 2nd, 4th, 6th, and 8th day repeated for 2-3 weeks.

AMH levels were assessed using the venous blood samples. Blood samples were placed in a serum separator tube and stored at -80°C in a laboratory-grade freezer until the sample size was complete.

AMH levels were measured before and after MTX therapy using the Electrochemiluminescence Immunoassay Analyzer (ECLIA) method (AMH Cobas Roche, Roche Corp. USA) and measured in ng/ml units. The results of AMH levels data were recorded into a spreadsheet. ß-hCG levels were measured quantitatively by the enzyme-linked immunosorbent assay (ELISA) method, with classifications of 1) <1,000; 2) 1,000-10,000; 3) >10,000 – 100,000; and 4) >100,000. Tumor sizes were measured using ultrasound examination results and classified as 0) tumor size <3 cm, 1) tumor size 3-4 cm, and 2) tumor size >4 cm.

Determination of the sample size was performed based on statistical calculations by setting a 95% confidence interval and 80% power test. Using the formula for determining the sample size for research on numerical analysis of the difference in the mean of the two groups in pairs, the sample size formula was as follows:

$$n_1 = n_2 = 2\left(\frac{(Z_a + Z_b)S}{X_1 - X_2}\right)^2$$

Description:
Za = standard deviation alph
Zb = standard deviation bet
S = combined standard deviation
$\mathbf{X_1\text{-}X_2}$ = the minimum difference in the average that is considered significant

$$S_c^2 = \frac{\left[s_1^2 \times (n_1 - 1) + s_2^2 \times (n_2 - 1)\right]}{n_1 + n_2 - 2}$$

Za, Zb = deviation value of Z obtained from the normal/standard distribution table for the confidence interval and selected parameters. Type 1 error was set at 5%, the hypothesis is two-way, so that Za = 1.96. Type 2 error was set at 20%, and Zb = 0.84 was obtained.

S = Standard Deviation based on previous research of 1.75 obtained from the combined S

d = X1-X2, namely the magnitude of the difference in the mean that is considered to be significant is 0.7.

Based on this formula, the value was entered into the sample size formula as follows:

$$n_1 = n_2 = 2\left(\frac{(1,96 + 0,84)\ 1,75}{0,7}\right)^2 = 49$$

Thus, the minimum number of subjects before and after chemotherapy was 49

samples. 1A loss-to-follow-up of 10% was added, making the final number of the research subjects was 49 + 4.9 = 53.9 54 subjects.

Statistical analysis was conducted to analyze the MTX chemotherapy on AMH levels, tumor size, and ß-hCG levels. In the case of normal data distribution, the paired t-test was used for data analysis; the Wilcoxon test as a fallback method was used in the case of abnormal distribution. The significance value was set at 0.05. All statistical data were analyzed using Statistical Product and Service Solutions (SPSS) version 24.0 for Windows (IBM Corp., USA).[16]

Results

This study was conducted from April to October 2020 at the Gynecological Oncology Department, Hasan Sadikin General Hospital Bandung. During the research, 54 participants met the inclusion criteria. Each participant was subjected to a blood sampling procedure to examine the serum AMH levels for further analysis.

The general characteristics of the research subjects included were body mass index, -hCG levels and tumor size. Based on theset characteristics, the mean age of low-risk GTD patients undergoing MTX chemotherapy was 31 years with the majority being in the age range of 31-35 years. Based on body mass index (BMI), the subjects were predominantly within normal BMI (57.3%), followed by obesity grade I (20.4%), overweight (16.7%), and obesity grade II with the least proportion (3.7%).

Venous blood samples were obtained from 54 participants fulfilling the inclusion criteria and AMH level, beta human chorionic gonadotropin (ß-hCG) level and tumor size were measured prior and post-chemotherapy.

A total of 54 low-risk women with GTD who met the inclusion criteria were evaluated. The mean age of participants was 31 years old, ranging from 31 to 35 years old. Table 1 shows the data on AMH and ß-hCG levels in low-risk GTD women before and after MTX chemotherapy. Our results showed a significant difference in serum AMH and ß-hCG levels before and after three cycles of MTX chemotherapy.

Table 2 shows the distribution of ß-hCG levels and tumor size with AMH in low-risk GTD women before and after three cycles of MTX chemotherapy. There were significant differences in serum AMH levels before MTX chemotherapy and after three cycles of MTX chemotherapy based on ß-hCG level tumor size.

A strong negative correlation was found between ß-hCG levels before chemotherapy and serum AMH levels after chemotherapy (r= -0.553, p<0.001), meaning that the higher the ß-hCG before chemotherapy, the lower the post-chemotherapy AMH serum levels.

There is a strong negative correlation between tumor size and serum AMH levels after chemotherapy (r= -0.577, p<0.001),

Table 1 Comparison of AMH and ß-hCG Levels In Low-Risk GTD Women Before and After MTX Chemotherapy

Variables	Before Chemotherapy	After Chemotherapy	P-value
AMH Level (ng/mL)*	2.88 ±0.98	2.06 ±0.92	<0.001*
ß-hCG level **	18.081,0 (1,2 – 1.000.000,0)	732,4 (0,3 – 886.300,3)	<0.001*

* Data presented as mean±SD.Paired T-test
** Data presented as Median (range). Wilcoxon test

Table 2 Distribution of Serum AMH Levels in Low Risk GTD Women Before and After MTX Chemotherapy

	N	AMH level before chemotherapy (ng/ml)	P-value*	AMH levels after Chemotherapy (ng/ml)	P-value*
ß-hCG levels					
<1000	15	3.53 ±0.75	<0.001	2.57 ±0.93	<0.001
1.000 – 10.000	10	3.24 ±0.62		2.46 ±0.53	
10.000 – 100.000	16	2.96 ±0.88		2.06 ±0.80	
>100,000	13	1.76 ±0.52		1.17 ± 0.62	
Tumor Size					
<3 cm	9	3.40 ±0.59	<0.001	2.46 ±0.46	<0.001
3-4 cm	25	3.36 ±0.80		2.49 ±0.82	
≥5 cm	20	2.06 ±0.76		1.34 ±0.74	

Data presented as mean±SD. *One Way ANOVA test

meaning that the larger the tumor size, the lower the serum AMH levels after chemotherapy.

Discussion

This study found a significant reduction in AMH levels following MTX chemotherapy in low-risk GTD patients with a difference of 0.82 ng/ml (SD: 0.40 ng/ml). This finding is supported by a prior study conducted in a Japanese population with low-risk GTD.[12] Another research conducted at Stanford University using juvenile idiopathic arthritis patients, also showed progressive reduction in AMH levels following MTX chemotherapy.[17] Measuring AMH levels before and after chemotherapy allows the detection of differences in ovarian damage caused by chemotherapy regimens and may help in the assessment of the long-term ovarian function.[18]

The impact of chemotherapy on the ovaries ranges from partial damage resulting in reduced fertility[18,19] to full damage with complete loss of primordial follicles[20], ovarian atrophy, and complete ovarian failure.[18,21] The extent of ovarian damage and the risk of infertility are dose-dependent and related to the age of the woman at the time of chemotherapy, with a greater risk of infertility and ovarian failure in patients of advanced reproductive age.[22,23] This is mainly due to the decrease in ovarian reserve that occurs naturally with age, as older women have fewer primordial follicles.[18,24] In addition, vascular damage and focal fibrosis in the ovarian cortex are other proposed mechanisms involved in chemotherapy-induced ovarian damage.[18,26]

Table 1 shows a significant difference from ß-hCG levels in participants before and after undergoing three cycles of MTX chemotherapy. ß-hCG level is a specific tumor marker for GTD that is easily measured quantitatively in the blood.[5] In GTD, ß-hCG level may persist or increase after mole evacuation, biopsy for the histology of choriocarcinoma, in the presence of invasive mole, or placental-site trophoblastic tumor (PSTT), or in the presence metastases.[27] As we found our results report that there is a decrease of ß-hCG levels, MTX agent chemotherapy is effective for low-risk

GTD treatment in Hasan Sadikin General Hospital.

A Cochrane meta-analysis study on the comparison of MTX chemotherapy with Actinomycin-D in low-risk GTD showed that Actinomycin-D has more severe side effects than MTX, despite its more superior efficacy.[4,11] Another study further supports the use of MTS by reporting MTX to be more cost-effective than Actinomycin-D and that Actinomycin-D did not reduce the use of multi-agent chemotherapy.[9,10]

We also found a significant negative correlation between ß-hCG levels before chemotherapy and serum AMH levels after chemotherapy. Therefore, the higher the levels of ß-hCG before chemotherapy, the lower the post-chemotherapy AMH level. MTX has an indirect effect in the form of MTX-PG accumulation in chorionic cells. ß-hCG is produced by malignant trophoblast cells. In patients with higher levels of ß-hCG, the accumulation of MTX-PG in the body is greater and causes longer-lasting side effects, which implies a more significant decrease in AMH levels.

Table 2 shows a similar finding that there is a significant negative correlation between tumor and serum AMH level after chemotherapy ($r = -0.583$, $p<0.001$). The larger the size of the tumor before chemotherapy, the lower the serum AMH level after chemotherapy. MTX-PG retention in tumor cells is longer. It has an indirect effect in the form of MTX-PG accumulation therefore, in GTD patients with larger tumor size, the accumulation of MTX-PG is more significant, resulting in a greater decrease in AMH levels. More research is still needed to determine whether the reduction in AMH levels would sustain after complete chemotherapy and the tumor has reduced in size.

There was a decrease in AMH and ß-hCG levels after three cycles of MTX chemotherapy in women with low-risk GTD. Tumor size and ß-hCG levels correlated with post-chemotherapy AMH results.

Limitation

There were limitations in this research, repetitive measure of AMH level was not fully assessed, therefore it is not certain whether the AMH level reduction may fluctuate during therapy. The AMH level of the participants included in this study was all evaluated after three courses of chemotherapy. Because the number of chemotherapy courses varies among GTD women, more research is needed to determine the level of AMH after full chemotherapy. In addition, further research is highly recommended to assess AMH reduction after chemotherapy completion to determine the sustainability of MTX chemotherapy.

Authors' contribution

IM, TH, AB, and BH designed the study and conducted the research. AR and JC monitored, evaluated, and analyzed the result of the study. IM, TH, AB, BH, AR, S and JC reviewed the article. All authors have reviewed the manuscript and have approved the last version of the manuscript.

Conflict of Interest

The authors of this study declare that there is no conflict of interest.

Ethical Issues

The Health Research Ethics Committee of Hasan Sadikin Hospital, Bandung, Indonesia has ethically approved this research with ethical clearance no LB.02.01/X.6/92/2020. After explaining the work process to the participants, those who wished to participate in this research were asked to sign the informed consent form.

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