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Adverse effects of methotrexate in gestational trophoblastic neoplasia treatment

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ABSTRACT

Objective: Identify the incidence and related factors of elevated transaminases in low-risk gestational trophoblastic disease patients treated with methotrexate. *Methods:* Prospective cohort. 163 patients were recruited with low-risk gestational trophoblastic disease, treated with methotrexate and folinic acid, from October 2019 to June 2020 at Tu Du hospital. Kaplan-Meier survival analysis is performed to determine the incidence of elevated transaminases over time. We apply Cox regression model in order to identify factors related to elevated transaminases and build a Nomogram to predict the risk of elevated transaminases. *Results*: The incidence of elevated transaminases is 28.83%. Out of 47 cases of elevated transaminases, 16 cases occurred after 1 cycle of treatment, 14 cases occurred after 2 cycles and 9 cases appeared after 3 cycles, and after 4, 5, and 6 cycles, there were only 4, 2, 1 cases of elevated transaminases, respectively. There were no cases after treatment cycle eighth and ninth. In patients with pre-treatment aspartate aminotransferase (AST)> 25 UI/L, the risk of elevated transaminases is 2.29 times higher than in patience with pre-treatment AST < 25 UI/L. Based on Cox regression model, we built a Nomogram with 4 variables, including age, body mass index (BMI), pre-treatment AST, and pre-treatment alanine aminotransferase (ALT). *Conclusions:* The overall incidence of elevated transaminases is 28.83%, usually occurred within the first 3 to 4 treatment cycle. Pre-treatment AST is related to elevated transaminases.

Keywords: elevated transaminases, GTN, MTX/FA, Nomogram

1. INTRODUCTION

The trophoblastic disease is a group of disorders characterized by abnormal proliferation of trophoblast, consists of two groups: gestational trophoblastic disease (GTD) and gestational trophoblastic neoplasia (GTN) (Goldstein & Berkowitz, 2007). In which, GTN includes: choriocarcinoma, placental-site trophoblastic tumor, epithelioid trophoblastic tumor, and invasive moles (Ngan et al., 2018). The incidence of thetrophoblastic disease varies among different regions of the world. Some countries with high rates of disease are Indonesia (10/1000 pregnant women), Mexico (4.6/1000), and Japan (2/1000) (Braga et al., 2019). North America and Europe have lower rates (Yuk et al., 2019). Vietnam has a relatively high incident rate of trophoblastic disease.

According to Pham Hao's research data (The Central Obstetrics Hospital), the rate of postmolar GTN was 20.2% (Hao, 2004). The report of the Department of Gynecological Oncology - Tu Du Hospital showed that the annual incident rate of postmolar GTN from 2015 to 2018 was 18.64%, 29.72%, 16.22%, and 19.5%, respectively.

Currently, Tu Du Hospital has been focusing on the management, treatment, and monitoring of most of the GTN patients in Southern Vietnam. Each year, the hospital received nearly 1,000 new molar pregnancies, and treated and followed up approximately 200 GTN patients. Most of these patients were low-incomed, low-educated, and dwelled in rural areas (Phan, Tran, 2015). Tu Du Hospital's current regimen for low-risk GTN is single-agent Methotrexate (MTX) or actinomycin D chemotherapy. However, due to the objective lack of actinomycin D, MTX/FA becomes the first choice regime.

MTX is a folate antagonist. It attaches to the dihydrofolate reductase, preventing the enzyme from converting to tetrahydrofolate acid and consequently inhibiting DNA, RNA, and protein synthesis (Olsen, 1991). Eventually, it causes cell death in the synthetic phase (S phase). Due to this mechanism, MTX effectively suppresses the growth of cancer cells. However, it also affects normal cell growth. The reported adverse effects of MTX include liver dysfunction, bone marrow suppression, gastrointestinal mucositis, pneumonia, nephrotoxicity, dermatitis, and some potentially life-threatening adverse events include hepatotoxicity, lung damage, and myelosuppression (Kang et al., 2010; Campbell et al., 2016; Görker et al., 2019). Currently, MTX/FA is the first choice regime in treating low-risk GTNat Tu Du Hospital (TuDu, 2019). However, there are lacking studies about the adverse effects of MTX in treating GTN at Tu Du Hospital. This issue is essential to aid physicians in advising the patients before initiating chemotherapy. Therefore, we performed this study to explore the frequency of the MTX chemotherapy regime's adverse effects in treating GTN.

2. METHODS

Research design

This is a prospective cohort study.

Research subjects

We recruited all GTN patients received MTX/FA regime and met the criteria at Tu Du Hospital from October 2019 to June 2020. We included the subjects with (1) Diagnosis of low-risk GTN; (2) Indicated to receive MTX/FA chemotherapy by the Chief/Deputy of the department or doctors with a certification of the Ministry of Health in utilizing chemotherapy in malignancies; (3) Agree to participate in the research. Subject's exclusion criteria were: (1) Diagnosis or suspicion of mental illness; (2) Previous abnormal liver or kidney function.

Sample size

The sample size was calculated according to the formula for estimating a proportion with absolute precision: $n= Z^2_{(1-\alpha/2)}P(1-P)/d^2$

In which, n: sample size, Z: confidence interval, P: anticipated prevalence or prevalence estimated from pilot study, and d: absolute precision (5%). Based on the rate of 8.5% of increased liver enzyme in Chalouhi GT's study (Chalouhi et al., 2009), our estimated sample size was 162.

Steps to conduct research

The study protocol was composed of 5 steps. Firstly, we detected and confirmed the low-risk GTN patients indicated to receive MTX/FA regime from the Department of Gynecological Oncology registry. Secondly, we invited the patients to participate in the study. The patients were clearly explained about the research objectives and methods. The participation or refusal to participate did not affect the patient's treatment. Thirdly, the data were collected by interviewing patients on an available questionnaire and recording the laboratory test results. Next, in case of patients dropping out of the follow-up visit, we contacted them by phone a maximum of three times. The patients were considered to be quitting if they did not return for a follow-up visit. Finally, we inputted and analyzed the data to complete the study report.

Data management and analysis

Data processing according to Stata 14 software. Data were reported as percentages, mean, and medians for each study variable. We performed the survival analysis applying the Kaplan-Meier method. Factors associated with increased liver enzymes were validated by the univariate and multivariate Cox regression model. The Nomogram model was established from the Cox multivariate regression analysis. The confidence interval of 95% and power of 90% were applied in the statistical analysis.

3. RESULTS

From October 2019 to June 2020, there were 172 cases diagnosed and treated with MTX/FA regimen. 163 of them were qualified and agreed to participate in the study (Table 1).

Table 1 Characteristics of our study population

Characteristics	Total (n)	Percentage (%)
Age		
≤40	124	76.07
>40	39	23.93
BMI (kg/m^2)		
≤25	151	92.64
>25	12	7.36
HbsAg		
Negative	153	93.87
Positive	10	6.13
Pre-chemotherapy AST (UI/L)		
≤25	130	79.75
>25	33	20.25
Pre-chemotherapy ALT (UI/L)		
≤25	129	79.14
>25	34	20.86
Parity		
Nulliparous	46	28.22
Primiparous	50	30.67
Multiparous	67	41.11
Miscarriage/Abortion		
Yes	69	42.33
No	94	57.67
History of previous molar pregnancies/GTD		
Yes	1	0.61
No	162	99.39
Blood type		
O	68	41.72
A	31	19.02
B	53	32.52
AB	11	6.75
Hysterectomy		
No	123	75.46
Yes	40	24.54
Previous chemoprophylaxis		
No	150	92.02
Yes	13	7.98

The patients' average BMI was 21.24, with the highest and lowest BMI was 38.7 and 15.4, respectively. 41.11% of the patients had given birth more than two times. Most of the patients were nulliparous, accounted for 28.22%. There was not much difference

regarding the history of abortion or miscarriage. There was one patient with a history of molar pregnancy. To determine the factors associated with increased liver enzymes, we classified the pre-chemotherapy AST value into two ranges of ≤25 UI/L and>25 UI/L. This was the 75th percentile in our study. There are 33 cases (20.25%) with pre-chemotherapy AST value of > 25 UI/L. Similarly, we classified the pre-chemotherapy ALT value into two ranges of ≤25 UI/L and>25 UI/L. There were 34 cases (20.86%) with pre-chemotherapy ALT of >25 UI/L. The rates of O, B, A, AB blood types were 41.72%, 19.02%, 32.52%, and 6.75%, respectively. All patients were received dilation and curettage therapy before treating with chemotherapy. Forty cases (24.25%) got over hysterectomy procedure. Thirteen cases (7.98%) received chemoprophylaxis before treatment.

Table 2 Rates of the increased liver enzymes in our study population

	Total (N=163)	Percentage (%)	95% CI
Solely increased AST	3	1.84	0.38-5.28
Solely increased ALT	12	7.36	3.86-12.51
Increased both AST and ALT	32	19.63	13.83-26.57
Increased AST or ALT	47	28.83	22.02-36.44
No increased AST nor ALT	116	71.17	63.56-77.98

There are three patients (1.84%) with solely increased AST value. Twelve patients (7.36%) only increased ALT value. Thirty-two patients increased both AST and ALT values (19.63%). Patients received the same treatment in case of an increase in AST or ALT values. Therefore, we considered the patients had increasing enzymes when their AST or ALT values increased. Consequently, there were forty-seven cases (28.83%, 95% CI 22.02-36.44%) of rising AST or ALT (Table 2).

Table 3 Frequency of increased liver enzymes cases over time in our study

Time (cycle)	Number of patients with increased liver enzymes (N= 47)	Percentage (%)	95% CI
1	16	9.82	6.13-15.52
2	14	18.65	13.42-25.28
3	9	24.96	18.88-32.57
4	4	29.96	22.82-38.87
5	2	34.48	25.86-44.98
6	1	40.44	27.94-55.92
7	1	52.53	31.51-76.59
8	0	52.53	31.51-76.59
9	0	52.53	31.51-76.59

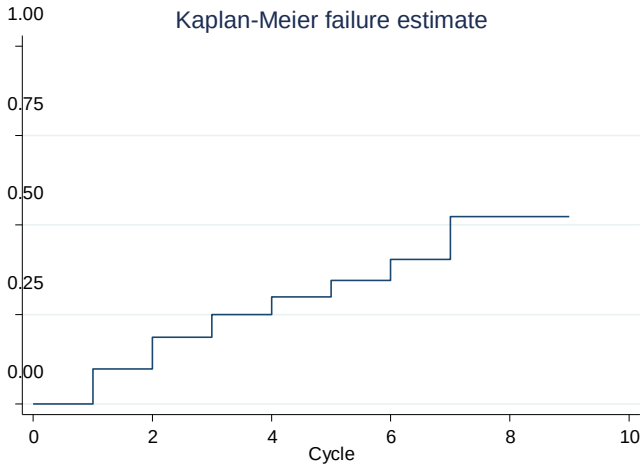

Figure 1 Frequency of increased liver enzymes accumulated over time

The highest frequency of increased liver enzymes occurred in the first 3-4 cycles, particularly after the first and second cycle. Specifically, there were 16, 14, 9, 4, 2, 1, and 1 case(s) of increased liver enzymes after the first to seventh cycle, respectively. Overall, up to 39/47 cases of increased liver enzymes occurred within three cycles (Table 3). 50% of BBTGBA patients in the study were recurrent within nine cycles (Figure 1).

Table 4 Multivariate analysis of factors associated with elevated liver enzymes

Factor	Risk time (cycle)	Number of patients without increased liver enzymes (N=163)	Number of patients with increased liver enzymes (N= 47)	Hazard Ratio	95% CI	P value
Age						
≤ 40	417	91(73.38)	33(26.62)	1		
>40	115	25(64.1)	14(35.9)	1.031	0.53-1.99	0.927
BMI						
≤ 25	406	111(73.51)	40(26.49)	1		
>25	26	5(41.67)	7(58.33)	2.216	0.94 -5.25	0.071
Pre-chemotherapy AST						
≤25	448	102(78.46)	28(21.54)	1		
>25	84	14(42.42)	19(57.58)	2.295	1.02-5.14	0.044
Pre-chemotherapy ALT						
≤25	438	101(78.29)	28(21.71)	1		
>25	94	15(44.12)	19(55.88)	1.637	0.73-3.66	0.262

After applying the univariate Cox regression analysis for fifteen factors, we put three factors with $p < 0.05$ and one factor (age) with $0.05 < p < 0.25$ in the multivariate analysis model. The result showed that the patient with a pre-chemotherapy AST value of >25 UI/L had 2.294 times higher risk of recurrence than the patient with pre-chemotherapy AST value ≤25 UI/L, $p < 0.05$ (Table 4).

4. DISCUSSION

Regarding the epidemiological characteristics, most of the patients in our study are in the reproductive age and from the outside of Ho Chi Minh City. The occupations were mostly worker, farmer, and house wife. Up to 30% of patients had a difficult economic condition. These characteristics are similar to other authors' studies when studying the population of GTD at Tu Du Hospital. Increasing in liver enzymes (AST or ALT) happened in 47 cases (28.3%). This result is consistent to the research result of Le Sy Phuong et al., (2009) (24.9%) due to the similarity of the study subjects' epidemiological characteristics. This rate is much lower than that of Woo Dae Kang et al., (2010) (52.54%). The reason might be because of the sample size. The sample size of Woo Dae Kang included only 59 patients receiving MTX/FA regimen. Our rate is higher than that of Chaloui et al., (2009) (8.5%), which is probably due to the differences in the study population. The study of Chaloui et al., (2009) conducted in France and had a different anthropological characteristics population. Nevertheless, the rate of increasing in liver enzymes varies significantly between studies.

When investigating the time of liver enzyme elevation, we found that most of them occurred within the first 3-4 cycles of treatment. Up to 39/47 instances of increasing in liver enzymes occurred within the first three cycles. Therefore, it can be seen that most of the elevation in liver enzymes occurred in the early time. When there was adoption in the following cycles, the rate of increasing in liver enzymes declined. No studies reported about these following values. Regarding factors related to elevated liver enzymes, patients with pre-chemotherapy AST value of >25 UI/L had a 2.294 times higher risk of an increase in AST/ALT than the group with pre-chemotherapy AST value of ≤25 UI/L (p-value< 0.05). Consequently, it seemed that patients with the low value of pre-chemotherapy liver enzymes would get tolerated with MTX/FA regime better. No studies reported about this issue.

We established the Nomogram model to determine the risk score of increasing liver enzymes for particular patients at a specific time in treatment. The Nomogram model was established by the multivariate Cox regression analysis model, including four factors:

BMI, age, pre-chemotherapy AST and ALT values. Here is an example: A28-year-old patient with stage I low-risk GTD is on MTX/FA regimen and has a pre-chemotherapy BMI of 25.5, and pre-chemotherapy AST and ALT value is 26 UI/Land 21 UI/L, respectively. The patient total risk score is 15. On a full score scale, the percentage of patients not having an increasing in liver enzymes are after the first to fourth cycle are 82%, 65%, 53%, and 44%, respectively (Figure 2).

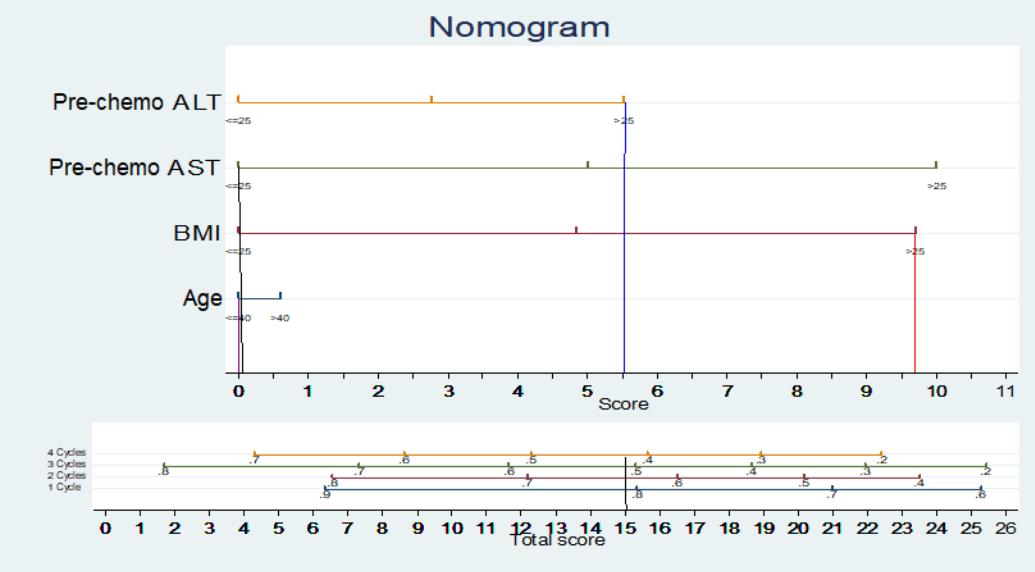

Figure 2 Application of Nomogram

Our study is relatively similar to other studies about the other undesirable effects that patients experience on MTX/FA treatment. Common side-effects were mouth ulcers, diarrhea, vomiting, and hair loss. Most of these effects were mild and moderate and did not affect the patients' treatment. Our research noted that most of these unpleasant effects occurred after the first and second cycle and decreased gradually in the following cycles. Patients were often instructed to drink plenty of water and improved nutrition without the need for specific treatment.

5. CONCLUSION

MTX/FA chemotherapy in the treatment of low-risk GTN patients is safe. The most common short-term side effect is the elevation of liver enzymes, which occurs mostly within the first 3-4 cycles, so special care should be taken at this stage for timely detection and management. More studies are needed to evaluate the long-term effects of MTX/FA chemotherapy in low-risk neoplasia and validate our Nomogram value to predict the risk of evaluating liver enzymes.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Informed consent

Written informed consent was obtained from all individual participants included in the study.

Ethics in research

The study was approved by the Ethics Council of UMP No. 475 / DHYD-HĐĐĐ, October 11th, 2019. The study was also approved by the Director Board of Tu Du Hospital in accordance with the Minutes No. 3537 / QD-BVTD Dec 12th, 2019.

Data and materials availability

All data associated with this study are present in the paper.

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