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Parameters of the glutathione system and thioredoxin in blood plasma and ascites and *GSTP1* Ile105Val gene polymorphism as factors of resistance to platinum-containing chemotherapy in ovarian cancer patients

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ABSTRACT

Background. Chemotherapy is one of the main types of treatment in ovarian cancer. Standard first-line treatment includes platinum drugs. Every fifth patient develops chemoresistance after platinum-containing first line therapy. Glutathione detoxification systems play an important role in platinum drugs utilization.

Aim. To assess the redox status of blood plasma and ascitic fluid in ovarian cancer patients before and after neoadjuvant platinum-containing chemotherapy (NACT).

Materials and methods. We determined the activity of the glutathione system and thioredoxin levels in blood plasma before and after NACT and in the ascitic fluid before NACT, and the presence of *GSTP1* gene polymorphism (Ile105Val (rs1695), Ala114Val (rs1138272) in 30 III–IV FIGO stage ovarian cancer patients. Patients were divided into 3 groups: NR – no relapse in 2 years after last chemotherapy course; R1 – relapse in less than 6 months; R2 – relapse in more than 6 months.

Results. We established an increase of the glutathione-transferase activity and a decrease of the GSH level in plasma after chemotherapy in R1 patients, and an opposite dynamic of glutathione-transferase and GSH in the R2 group. Thioredoxin level in plasma of all patients was lower than in the control group; differences in levels between groups were not statistically significant. *GSTP1* 105Val allele was more frequently present in patients than in the control group, and more frequently in R2 than in R1.

Conclusion. The increase in plasma glutathione-transferase and glutathione-reductase levels can be a prognostic marker of early relapse. Thioredoxine dynamics do not correlate with the chemotherapy response. The presence of the *GSTP1* 105Val allele is a risk factor for ovarian cancer development, but a protective factor against early relapse.

Key words: ovarian cancer, ascites, chemoresistance, glutathione system, *GSTP1* gene polymorphism.

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Параметры глутатионовой системы и тиоредоксина в плазме крови и асците и полиморфизм гена *GSTP1* Ile105Val как факторы резистентности к платиносодержащей химиотерапии у больных раком яичников

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РЕЗЮМЕ

Введение. Химиотерапия является одним из основных видов лечения распространенного рака яичников (РЯ). У каждой пятой пациентки развивается химиорезистентность после платиносодержащей терапии первой линии. Система детоксикации глутатиона играет важную роль в утилизации платиновых препаратов из опухолевых клеток.

Цель. Оценить окислительно-восстановительный статус плазмы крови и асцитической жидкости у больных РЯ до и после неоадъювантной платиносодержащей химиотерапии (НАХТ).

Материалы и методы. Мы определили активность глутатионовой системы и уровень тиоредоксина в плазме крови до и после НАХТ и в асцитической жидкости до НАХТ у 30 пациентов на III–IV стадиях (по FIGO) рака яичников. Пациенты были разделены на три группы: БР – без рецидивов в течение 2 лет после завершения химиотерапии; Р1 – рецидив заболевания в течение 6 мес после завершения химиотерапии первой линии; Р2 – рецидив после 6 мес от момента завершения химиотерапии первой линии.

Результаты. Установлено увеличение активности GT и снижение уровня GSH в плазме после химиотерапии у пациентов с Р1, а также противоположная динамика GT и GSH в группе Р2. Уровень тиоредоксина в плазме у всех пациентов был ниже, чем в контрольной группе; различия в уровнях между группами не были статистически значимыми. Аллельный вариант 105Val гена *GSTP1* выявлялся с более высокой частотой у пациентов с РЯ, чем в контроле, и чаще в группе Р2, чем у Р1.

Заключение. Повышение активности GST и GR в плазме больных РЯ может быть прогностическим маркером раннего рецидива. Динамика тиоредоксина не коррелирует с ответом на химиотерапию. Присутствие аллеля 105Val в гене *GSTP1* является фактором риска развития рака яичников, но защитным фактором против раннего рецидива.

Ключевые слова: рак яичников, асцитическая жидкость, химиорезистентность, глутатионовая система, полиморфизм гена *GSTP1*.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The increased glutathione system activity underlies the resistance to platinum-containing chemotherapy (CT) in ovarian cancer (OC), among other causes. Binding to SH-groups of glutathione inactivates cisplatin, with mainly glutathione-S-transferases (GST) providing the neutralization [1]. Platinum-resistant cells demonstrate a higher activity of GST in the cytosol compared with the original platinum-sensitive SKOV3 and SGC7901 cells, and the GSTP inhibition increases the cytotoxicity of platinum drugs by 4 times [2]. GST plays a role in drug resistance by providing the direct detoxifying effect and the MAP kinase pathway inhibition. GST modulates the tumor response to platinum-containing CT in OC [43]. GST activity in ovarian cancer ascites negatively correlates with the sensitivity to platinum drugs and positively correlates with the risk of recurrence. The expression of the glutathione-dependent enzymes genes reflects the adaptive antioxidant potential and can take part in drug resistance development [4].

The limited data are available on the thiol cell detoxification systems, represented by thioredoxin (Trx) and glutaredoxin, the regulators of the redox potential, cell proliferation, and DNA repair. Some authors propose that thioredoxin contributes to the formation of doxorubicin and cisplatin resistance, protecting cells from the oxidative stress and inhibiting the apoptosis through protein kinases ASK1 and JNK1 [5].

The aim of the study was to assess the parameters of the glutathione system and thioredoxin in blood plasma and ascites and polymorphism of the *GSTP1* Ile105Val gene as factors of chemoresistance in patients with advanced ovarian cancer.

MATERIALS AND METHODS

The study included 30 patients (median age 62 years; lower quartile – 45, upper quartile – 65) with verified OC presenting with ascites (performance status according to ECOG – 0–2, life expectancy at least 6 months). The patients were treated with 2–4 cycles of the neoadjuvant chemotherapy (NACT) according to the AP regimen (cisplatin 75 mg/m² and doxorubicin 40 mg/m² intravenously in the 1st day every 3 weeks) and, subsequently, cytoreductive surgery and adjuvant chemotherapy. Ascitic fluid (AF) for analysis was taken before starting chemotherapy. The cell-free fraction was collected after centrifugation at 1,500 rpm for 10 min. With dynamic observation, all patients were divided into groups: NR – no relapse; R1 – early relapse, relapse-free period up to 6 months; R2 – late relapse, relapse-free period from 6 to 12 months. In the plasma of patients before treatment and after NACT and AF before treatment, the activity of the glutathione system components was determined: glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPO), and the level of reduced glutathione (GSH) [6, 7]; thioredoxin (Trx) level was evaluated by ELISA (Cloud Clone Corp., USA). Genomic DNA for the analysis of *GSTP1* gene polymorphisms Ile105Val (rs1695), Ala114Val (rs1138272) was isolated with a "DNA express blood" kit (NPF Litech, Moscow). Genotyping of the samples was performed by allele-specific real-time PCR with Taq-Man probes (Syntol, Moscow). The control group consisted of 20 apparently healthy women (median age 52, lower quartile 45; upper quartile 58). Quantitative data are presented as median and lower and upper quartiles. Due to the abnormal distribution in the groups, the nonparametric Kruskal – Wallis test was used to describe the statistical differences (the differences were considered significant at $p \leq 0.05$). The frequencies of the *GSTP1* Ile105Val gene polymorphism, as well as the correspondence of the distribution of the observed genotype frequencies to the theoretically expected ones from the Hardy – Weinberg equilibrium, were checked using the $\chi 2$ test. To assess the relative risk of developing an event, the OR (odds ratio) value and confidence intervals were calculated using an on-line calculator in case-control studies (http://gen-exp.ru/calculator_or.php). Statistical data processing was carried out using the Statistica 13.0 software package.

RESULTS

The activity of GST statistically significantly differs between the groups of OC patients, depending on the time to the recurrence. Plasma GST activity in patients of the R1 group is several times higher than the values GST in the control, R2 and NR groups both before and after NACT (Fig. 1,*a*). The same dynamics exists in the AF: the early relapse is associated with high enzyme activity before treatment (Fig. 1,*b*). The high detoxification potential of GST may reduce the effectiveness of platinum-containing NACT by conjugating the drug, along with a decrease in free GSH. After NACT, the enzyme activity in all studied groups slightly decreases, while remaining elevated in the R1 group. The decrease in the plasma GST activity after NACT is associated with a longer relapse-free period.

The main function of GR is to maintain glutathione in the reduced form, which conjugates with exogenous toxins. The change in GR activity in the plasma of OC patients is similar to the dynamics of GST in plasma (Fig. 1,*a*), which suggests a decrease in the enzymatic recovery of GSSG after NACT.

In the R1 group, the lowest GSH values are found in blood plasma in comparison with all other groups studied (Table 1). In the NR group, low plasma GSH values

are observed before treatment, and a sharp increase (by 3.5 times) happens after NACT, which possibly reflects an increase in the antioxidant status and may act positively, preventing the formation of resistance to doxorubicin and cisplatin.

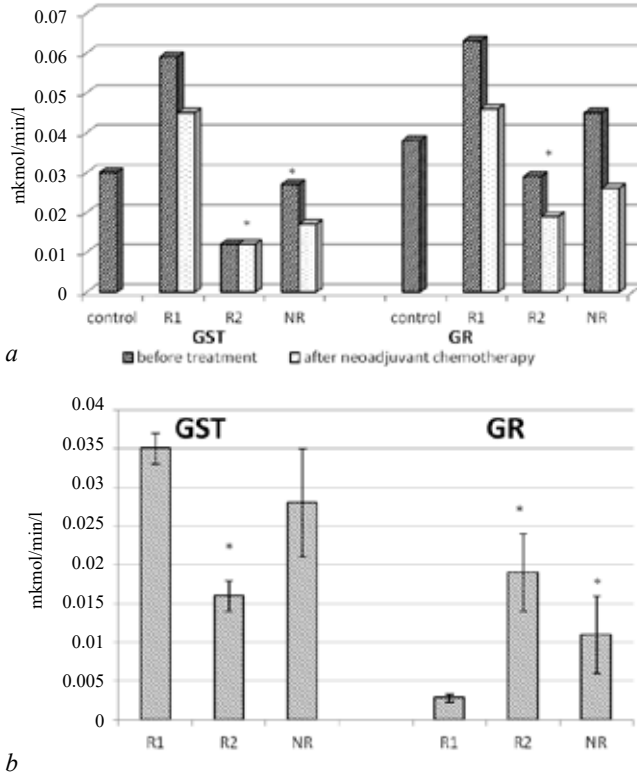


Fig.1. Level of GST and GR in plasma (*a*) and ascitic fluid (*b*), depending on the duration of the relapse-free period: * marked data are significantly different from the group R1

The activity of GPO, utilizing hydrogen peroxide, is lower than the control in blood plasma in all groups and does not differ significantly (Table 1).

Ascites sampling may reveal additional factors to clarify the molecular biological "portrait" of the ovarian tumor [8]. We found that the R1 group had the lowest GSH values in AF, while the maximum level was in the R2 group (Table 1). Thus, an increase in GST and a decrease in GSH in plasma after NACT may be markers of early relapse. In contrast, low GST activity and high GSH levels in plasma and ascites occurred in the group of NR patients.

Trx may play a role in the mechanisms of antitumor drug resistance; this effect is tissue-specific and depends on the microenvironment [9]. We found that the Trx level in blood plasma, decreased in all groups compared to the control, did not differ significantly before and after platinum-containing chemotherapy (Fig. 2).

Tumor sensitivity to cisplatin is determined by the activity of detoxification enzymes, which include GST, and the activity depends on gene polymorphism [10]. When comparing two groups, patients with OC and controls, we found that the presence of a functionally weakened *GSTP1* allele (genotype Ile/Val or Val/Val) in the genotype is a risk factor for OC (OR = 1.82; 95% CI 1.1–2.8; $p = 0.035$). The *GSTP1114Val* allele is associated with a decrease in the functional activity of the enzyme and is also more common in OC patients than in the controls (19% versus 5.5%; OR = 3.20; 95% CI 1.5–6.8; $p = 0.023$). In the R1 group, compared with the R2 patients, the Ile/Ile *GSTP1* genotype is more common (OR = 4.30; 95% CI 1.25–14.81; $p = 0.034$).

Table 1

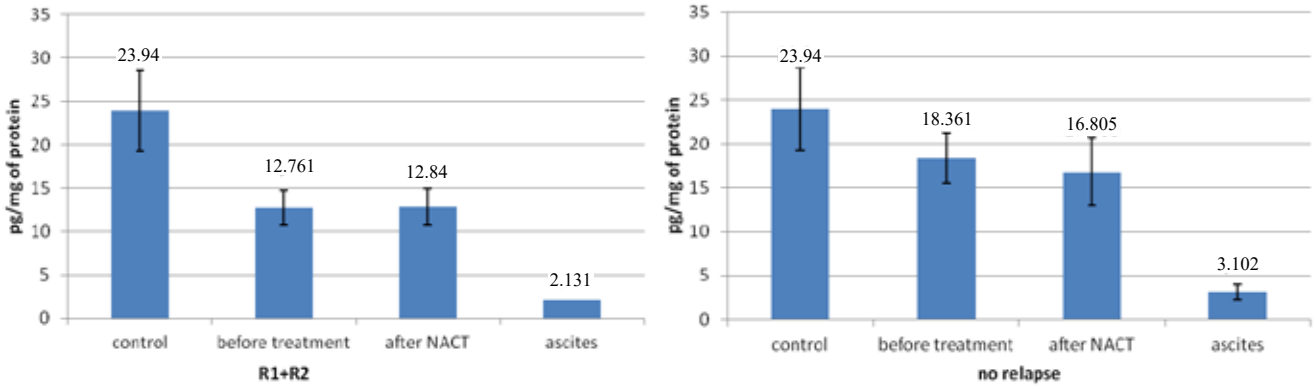
GSH and GPO levels in plasma and AF in patients with advanced OC before and after NACT, *Me* (Q_1–Q_3)

Parameters	GSH, mmol/l			GPO, μmol/min/l		
	R1, $n = 12$	R2, $n = 8$	NR, $n = 10$	R1, $n = 12$	R2, $n = 8$	NR, $n = 10$
Plasma of primary patients	19.72 (15.64–22.44)	72.8 (54.0–95.5) $p = 0.00005$	25.16 (23.8–30.2) $p = 0.003$	9.075 (3.675–11.835)	7.905 (4.372–12.278) $p = 0.653$	11.048 (10.83–11.67) $p = 0.101$
Plasma of patients after completing NACT	28.9 (14.96–44.4)	47.5 (27.3–74.45) $p = 0.219$	105.5 (53.16–121.00) $p = 0.0006$	10.477 (10.41–11.07)	10.695 (6.405–17.10) p=0.477	8.28 (8.1–9.24) $p = 0.619$
Ascitic fluid of OC patients before treatment	19.72 (17–27.2)	101 ***(93–112.5)*** $p = 0.003$	32 20.4–98.5 $p = 0.160$	19.2 (12.3–30.3)	7.073 (4.22–9.82) $p = 0.037$	19.83 (15.75–25.62) $p = 0.802$
Control	80.72 (76.5–82.3)			52.01 (50.5–54.0)		

DISCUSSION

Ovarian carcinogenesis provokes a prooxidant state, both in the tumor and in the adjacent normal tissues, and the subsequent chemotherapy aggravates this condition. In the late stages of OC, depletion of antioxidant resources occurs. The glutathione system in the blood plasma can move to a higher level of functioning and provide protection of macromolecules from reactive oxygen species. We describe such observation in the group of patients with a long relapse-free period. Conversely,

the decrease in the antioxidant proteins favors the survival of a tumor and the metastasis development. We observed significantly lower values of GSH and Trx levels in plasma in the group of OC patients with relapses in comparison to the NR group. The glutathione system plays a dual role in the process of carcinogenesis [11].



a *b*

Fig. 2. Level of thioredoxin in plasma and ascitic fluid of patients with OC before and after NACT with early and late relapse (R1 + R2) (*a*) and without relapse for 2 years (NR) (*b*)

On the one hand, its low activity disrupts the inactivation of carcinogens. The decrease in the concentrations of GSH and GSH-dependent enzymes in plasma and ascites are observed with tumor progression. Earlier in the experiment, we established a tendency towards a decrease in lipid peroxidation and oxidative modification of proteins in ascites with the progression of OC [12]. On the other hand, GSH and Trx in tumor reduce the cytotoxic effect of cisplatin; low levels of Trx in the cytoplasm of OC cells are associated with an increase in progression-free survival [13]. The observed dynamics of Trx levels in plasma and ascites needs further studying of the Trx role in the formation of OC cells clones with a high antioxidant status.

GSTP activity differs depending on the substrate; with the GSTP1105Val allele, the overall survival in OC patients is reduced after the platinum-containing chemotherapy [14]. Thus, it can be assumed that overexpression of the GSTP gene determines the resistance of OC cells to platinum-containing CT.

CONCLUSION

The platinum-containing NACT has a significant effect on the functional state of the glutathione system in the plasma of OC patients. The patients of the early relapse group have decreased GSH and Trx levels, and increased activity of GST and GR in plasma after NACT. In patients with no relapse in 2 years, an increase in the antioxidant status of plasma is observed: the levels of GSH and thioredoxin are increased, and the activity of GST is decreased. In general, the system of glutathione and glutathione-dependent blood plasma enzymes dynamically changes its profile during ovarian carcinogenesis and may be used for assessing individual sensitivity to platinum-containing chemotherapy, as a prognostic marker for early relapse. The presence of the Ile105Val allele in the GSTP1 gene is a risk factor for the development of ovarian cancer, but a protective factor against early relapse.

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Authors contribution

Dolgova D.R. – conception and design of the study, interpretation of the data. Antoneeva I.I. – selection of a clinical base for analysis. Fedotova A.Yu. – analysis and interpretation of the data. Gening S.O. – substantiation of the manuscript and revision of the content. Abakumova T.V. – analysis and interpretation of the data. Gening T.P. – final review of the manuscript for publication.

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