MONITORING OF CHEMOTHERAPY SUCCESSFULNESS OF PLATINA/TAXOL CHEMOTHERAPY PROTOCOL BY USING DETERMINATION OF SERUM UROKINASE PLASMINOGEN ACTIVATOR (UPA) AND SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (SUPAR) IN PATIENTS WITH OVARIAN CARCINOMA FIGO II AND III STAGE

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Abstract

In about 70% of cases, ovarian carcinoma has been diagnosed at an advanced stage. Invasion and metastasis of solid tumors request protease activity resulting in basal membrane destruction and surrounding matrix. In that process, urokinase plasminogen activator (uPA) and its receptor, urokinase plasminogen activator receptor (suPAR) play a key role, that via plasmin activation lead to basal membrane and matrix degradation in surrounding of the tumor, enable to its invasion and metastasis. Determination of serum concentration of those tumor markers can be useful in preoperative as well as in postoperative period. Their serum concentrations in ovarian cancer patients may help in good monitoring of remission or progression during chemotherapy treatment. In late 1950s and ear1y 1960s, when it was found out that malignant ovarian tumors were chemosensitive, their chemotherapy treatment has begun. In the beginning it was used only mono-therapy, and by discovering new cytostatics it was replaced by poly-chemotherapy. Now days, in the therapy of advanced stages of ovarian carcinoma combination of cisplatine or carboplatine with paclitaxel is considering as standard treatment. Aim of this study was to determine serum uPA, suPAR and CEA in FIGO II and III patients with different histological type (serous, mucinous, clear cell tumor) before and after PT chemotherapy protocol during following three cycles. In this prospective study we have analyzed 17 patients with ovarian carcinoma, those have been after surgery treated by chemotherapy. Serum levels of uPA and suPAR have been determined by ELISA-test (Imubind uPA, Imubind uPAR, American Diagnostica), and CEA by OPUS Imunoassay method. Results of this study have shown that uPA, suPAR and CEA met criteria for prognostic markers for monitoring of successfulness of platina/taxol chemotherapy protocol for serous, mucinous and clear cell tumor FIGO II and III stage of ovarian carcinoma. In case of PT chemotherapy protocol suPAR was better prognostic marker for monitoring of chemotherapy successfulness (Pearson coefficient 0,9 do 1,0; p<0,00l) than uPA (Pearson coefficient between 0,86 and 0,92; p<0,02) and CEA (Pearson coefficient 0,5 do 0,89; p<0,04).

KEY WORDS: ovarian carcinoma, chemotherapy, urokinase plasminogen activator-uPA, urokinase plasminogen activator receptor-suPAR

Introduction

Ovarian carcinomas make 4% out of all malignant tumors in women, and as a death cause in the same population are on 4th place (1). Despite aggressive treatment applying late years, a five-year survival rate is se 30% (2). However, a five-year survival rate has been significantly increasing (about 90%), even disease is discovered and treated in early stage (3). Malignant ovarian epithelial tumors consist of 90% out of all malignant ovarian tumors (4). In most of the patients malignant ovarian tumors has been diagnosed at advanced stage (5), because of anatomic position of ovarium and poor clinical symptoms. Just 15 out of 2000 well-known enzymes are useful in diagnostics and monitoring of development of ovarian carcinomas. Plasmin is an enzyme which is formed by cleavage of plasminogen and plays important role in proteolytic degradation surrounding intercellular matrix, allowing invasion and metastasis. Over few late years, it has been found an association between urokinase plasminogen activator (uPA) and its soluble receptors (suPAR) and development of different malignant tumors, among them ovarian carcinomas (6, 7, 8, 9, 10). Beside in preoperative diagnostics of malignant tumors, uPA and suPAR may be determined in postoperative diagnostics and be useful in monitoring of remission and progression of the disease, and successfulness of certain chemotherapy protocol, as well. In late 1950s and 1960s, when it has been found that malignant tumors of the ovarium are chemosensitive, the application of chemotherapy had been began. In the beginning, just mono-chemotherapy has been applied. After discovering new cytostatics, mono-therapy has been replaced by poly-chemotherapy. In late 1970s it has been discovered very powerful drug platina, which is today the base of all successful protocols. Successfulness of the treatment by chemotherapy depends on administered dose (11). There are a numerous chemotherapy protocols that have different response rate with adverse effects such as alopecia, vomiting, myelosuppression, nephrotoxicity and etc. Standard chemotherapy application is cyclic repeating every 21-28 days, in order to recovering immunobiological system of the body. U patients with early stage of ovarian carcinoma the chemotherapy protocol is based on platina. In the treatment of advanced stage of ovarian carcinoma combination of cisplatina or carboplatina with paclitaxel is considered as standard treatment (12, 13). Aim of this study was to determine serum uPA, suPAR and CEA in FIGO II and III patients with different histological type (serous, mucinous, clear cell tumor) before and after PT chemotherapy protocol during following three cycles.

Patients and Methods

In this prospective study we have analyzed 17 patients with ovarian carcinoma, those have been after surgery treated by chemotherapy and intraoperatively staged according FIGO classification. Serum levels of uPA and suPAR have been determined by ELISA-test (Imubind uPA, Imubind uPAR, American Diagnostica), and CEA by OPUS Imunoassay method. Eight out of 17 patients were FIGO II, and nine were FIGO III stage. According to histological type nine of them had mucinous, five clear cell tumor and three serous type. Chemotherapy was administered every three weeks in one dose (Platina 100 mg/m2) and (Taxol 135 mg/m^2). Biological material was serum taken from the patients immediately before the application of certain chemotherapy cycle and always under same conditions by experienced medical staff in the morning between 10 and 11 am. Blood sample 4 ml plus 0,5 ml Na-citrate was immediately centrifuged at 3000 rpm in 15 min at room temperature. The samples were preserved at -20^{o}C until use. In this study were included just those patients who had toxicity rate less then 2. All patients were introduced about this study and they agreed with it. Control group make patients prior chemotherapy application, and experimental one the same patients after chemotherapy application. In serum of those patients we have determined level of uPA, suPAR and CEA during three following chemotherapy cycles. Process of remission or progression for measurable and immeasurable lesions has been monitored according definition of objective chemotherapy successfulness (Table 1) by CT (Toshiba 600 HQ; SOMATOM AR STAR-Siemens), trans-vaginal ultrasound (Toshiba

	Measurable process	**Non-measurable process**
Complete successfulness	No disease at least 4 weeks	No disease at least 4 weeks
Partial successfulness	Decrease of tumor mass at least for 50%	Decrease of tumor mass at least for 50% or more
No successfulness	No decrease of tumor mass for 50%, but there is no increase of lesions for 25%	No significant changes at for 4 weeks
Process progression	Increase one or more lesions for 25% or appearance of new ones	appearance of new lesions or increase of lesions that already exist

TABLE 1. Definitions of objective successfulness of chemotherapy

Sonolyer-L SAL- 77 B) and laboratory findings (Whole blood, ALT, AST, creatinine, urea, urine and ERS). Patients according to age have been divided into five groups: <41, 41-50, 51-60, 61¬-70 and >70 years. Urokinase plasminogen activator (uPA) has been determined in serum by ELISA kit Imubind uPA #894 (American Diagnostica, Greenwich, CT, USA). This test is very sensitive and allows possibility for detection of uPA even very low level such as 10 pg/ml. Serum level of uPA has been determined by BIORAD MICROPLATE MENAGER with program IZODATA. Serum level of uPA is quantified by measuring of level of absorption at 450 nm and comparing with values from standard curve. urokinase plasminogen activator receptor-suPAR has been determined by ELISA kit Imubind suPAR #893 (American Diagnostica, Greenwich, CT, USA). This test is very sensitive and allows possibility for detection of suPAR even very low level 0,1 ng/ml. Data have been analyzed by Student's *t-test* and Pearson's correlation test.

RESULTS

In this study 17 patients (8 FIGO II and 9 FIGO III stage) who had pathohistologicaly verified ovarian carcinoma and who have been treated by Platina/Taxol chemotherapy protocol after surgery. In serum of the patients with these stages, mucinous type, it has been found statistically significant difference between level of uPA after (0,40 ±0,19 ng/ml) Platinal/Taxol chemotherapy application for certain cycles comparing with level of uPA prior to application (0,69±0,23 ng/ml) (t-test- 4,78; p<0,05) (Figure 1).

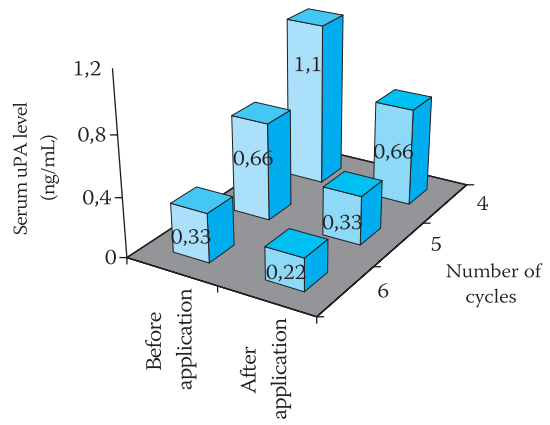

FIGURE 1. Serum uPA level in patients FIGO IIa+b, mucinous type, before and after application of PT chemotherapy protocol

Decrease uPA serum level of the patients with FIGO IIc mucinous type (Figure 2) after PT chemotherapy protocol application (0,36±0,11 ng/ml) was statistically significant comparing with that one prior to application (0,73±0,15 ng/ml) (t-test: 5,97; p<0,04).

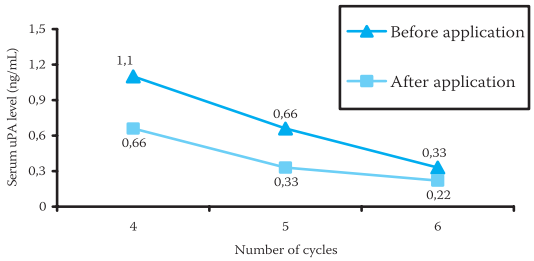

FIGURE 2. Serum uPA level in patients with ovarian carcinoma FIGO IIc, mucinous type, before and after application of PT chemotherapy protocol

In serum of the patients with FIGO IIc stage type clear cell tumor (Figure 3) it has been found statistically significant decrease of uPA level after application of chemotherapy PT protocol (0,40±0,04 ng/mL) comparing control group (0,73±0,06 ng/ml) (t-test: 5,20; p<0,04).

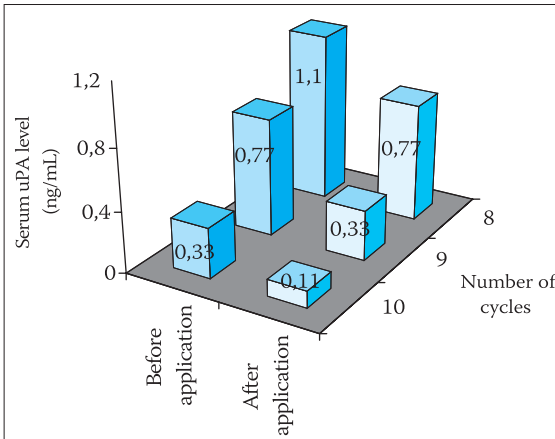

FIGURE 3. Serum uPA level in patients with ovarian carcinoma FIGO IIc, clear cell tumor, before and after application of PT chemotherapy protocol

It has been observed that in patients with FIGO II stage who had statistically significant decrease of uPA level after PT chemotherapy protocol application u comparing control group, they had better quality of life during chemotherapy (Karnofsky scale score 80-90%). According to analysis of objective condition of successfulness of chemotherapy, it can be said that PT chemotherapy application for mucinous and clear cell tumor was completely successful. Analyzing serum uPA levels after PT chemotherapy protocol and comparing them in patients with different histological types, we have noticed that uPA

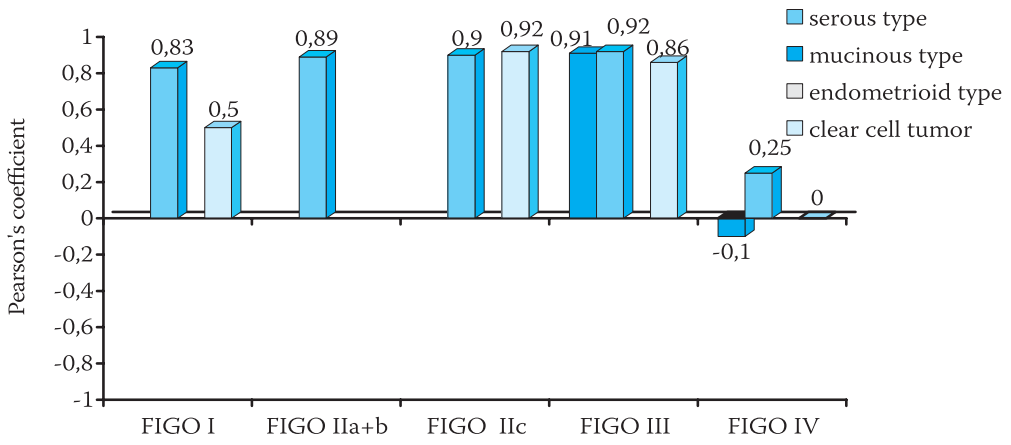

FIGURE 4. Correlation serum uPA level with FIGO stage and hystological type of ovarian carcinoma in patients treated by PT chemotherapy protocol

is good indicator for monitoring of successfulness of chemotherapy protocol for mucinous and clear cell tumor (Pearson's coefficient 0,89-0,92) (Figure 4). In patients with FIGO III stage it has been found statistically significant decrease of uPA level after application of chemotherapy PT protocol comparing control group with good score for quality of life (Karnofsky scale score 80%). According to analysis of objective condition of successfulness of chemotherapy for patients whom was applied PT chemotherapy protocol and who had mucinous type of ovarian carcinoma, it can be said that successfulness was partial. Patients with clear cell tumor had stable disease.

In the same patients serum levels of suPAR and CEA have been determined under same conditions. For patients with FIGO IIa and FIGO IIb and FIGO IIc stage, it has been observed statistically significant decrease of suPAR after PT chemotherapy protocol application. Karnofsky scale score was between 80 and 90% with complete successfulness. Analyzing serum suPAR levels after PT chemotherapy protocol and comparing them in patients with different histological types, we have noticed that suPAR is good indicator for monitoring of successfulness of chemotherapy protocol for mucinous and clear cell tumor (Pearson's coefficient for PT pro-

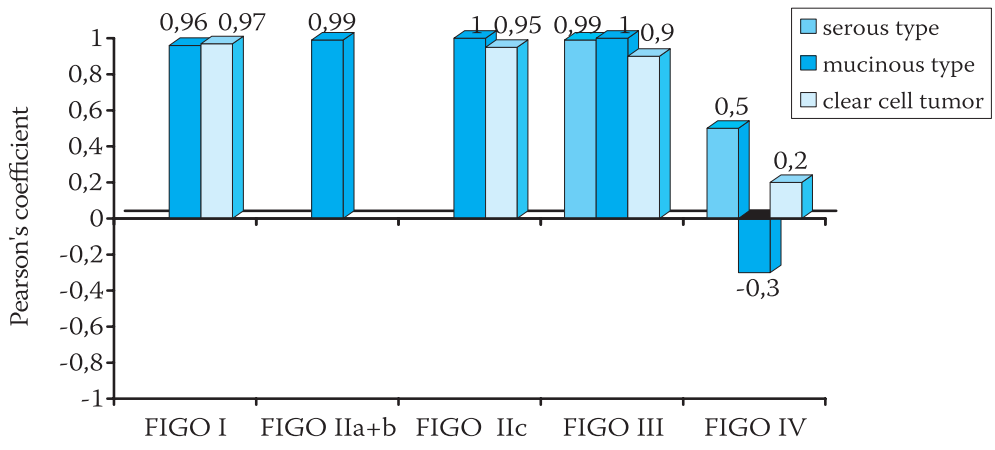

FIGURE 5. Correlation of suPAR with FIGO stage and hystological type of tumor in patient with ovarian carcinoma treated by PT chemotherapy protocol

TABLE 2. Correlation serum level of uPA, suPAR and CEA with FIGO stage and hystological type of ovarian carcinoma in patients treated by PT chemotherapy protocol

FIGO stage / Marker	I		IIc		IIa+b		III			IV	
	m	cct	m	cct	m	s	m	cct	s	m	cct
uPA	0,83	0,5	0,90	0,92	0,89	0,91	0,92	0,86	-0,1	0,25	0,0
suPAR	0,96	0,97	1,0	0,95	0,99	0,99	1,0	0,90	0,5	-0,3	0,2
CEA	0,5	-0,65	0,88	0,5	0,85	0,5	0,89	0,69	0,54	0,62	0,5

(s-serous; m-mucinous; cct-clear cell tumor)

tocol-mucinous type FIGO IIa+b 0,99, mucinous FIGO IIc 1,0 and clear cell tumor 0,95) (Figure 5). In patients with FIGO III stage it has been found statistically significant decrease of suPAR level after application of chemotherapy PT protocol comparing control group with good score for quality of life (Karnofsky scale score 80%). According to analysis of objective condition of successfulness of chemotherapy for patients whom was applied PT chemotherapy protocol and who had serous and mucinous type) it can be said that successfulness was partial, Patients with clear cell tumor had stable disease. Analyzing serum suPAR levels after PT chemotherapy protocol and comparing them in patients with different histological types, we have noticed that suPAR is good indicator for monitoring of successfulness of chemotherapy protocol for serous, mucinous and clear cell tumor (Pearson's coefficient for PT protocol -serous 0,99; mucinous 1,0; clear cell tumor 0,90). In Table 2 are shown Pearson's coefficient values for FIGO stages and histological types in patients who have been treated by PT chemotherapy protocol.

Discussion

Kuhn et al. (14) have reported that serum level of uPA in patients with FIGO III stage of ovarian carcinoma has shown high rate of negative correlation with a five-year survival rate meaning the higher serum level of uPA above 0,92 ng/ml the worse prognosis ($p<0,01$). Similar results have been reported by Pujade-Lauraine et al. (15), Casslen et al. (16) and Schmalfeldt et al. (17) have found that uPA could be good indicator for monitoring of patients with FIGO II and FIGO III stage of ovarian carcinoma. Since suPAR is relatively new marker, so far it has not been determined its importance in monitoring of successfulness of Platina/Taxol chemotherapy protocol after surgery. Comparing serum suPAR level after application of this protocol it has been found that suPAR is good indicator of successfulness for serous, mucinous and clear cell tumor (Pearson's coefficient was 0,95 and 1,0). Authors investigating fibrinolytic markers (18) emphasize need further research some marker that does not belong to fibrinolytic system in order to correlate it with values of uPA and suPAR. Because of that in this study we have determined serum CEA level as one of the routine marker preoperatively in diagnostics of ovarian carcinoma and postoperatively in monitoring of disease course and successfulness of chemotherapy (5). We have found suPAR to be better prognostic marker for successfulness than uPA in patients treated by PT chemotherapy protocol (Pearson's coefficient between 0,9 and 1,0; $p<0,001$) comparing to uPA (Pearson's coefficient between 0,86 and 0,92; $p<0,02$) and CEA (Pearson's coefficient between 0,5 and 0,89; $p<0,04$).

Conclusion

Analyzing serum level of tested markers in patients after PT chemotherapy application and comparing them with histological type and FIGO stage it has been found that suPAR and uPA are good indicators of successfulness of this chemotherapy protocol for serous, mucinous and clear cell tumor FIGO II and FIGO III, and CEA for mucinous type.

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