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Original Research Article

The significance of reduced glutathione and glutathione S-transferase during chemoradiotherapy of locally advanced cervical cancer

Lina Daukantienė *, *Birutė Kazbarienė*, *Konstantinas Povilas Valuckas*, *Janina Didžiapetrienė*, *Aurelija Krikštaponienė*, *Eduardas Aleknavičius*

National Cancer Institute, Vilnius, Lithuania

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ABSTRACT

Background and objective: To determine changes in reduced glutathione (GSH) and glutathione S-transferase (GST) during neoadjuvant chemotherapy followed by concurrent chemoradiation for patients with stage IIB–IIIB cervical cancer, and to evaluate their significance to the efficacy of the treatment.

Materials and methods: According to the prospective phase II study protocol, 36 patients with stage IIB–IIIB cervical cancer were enrolled. A short course of intensive weekly neoadjuvant cisplatin and gemcitabine chemotherapy followed by concurrent weekly cisplatin and gemcitabine-based chemoradiation was administered. Blood samples for GSH, GST analysis were collected and analyzed before the start of the treatment, after neoadjuvant chemotherapy, and after the end of the chemoradiation.

Results: A statistically significant increase in the concentration of GSH after neoadjuvant chemotherapy was identified. After chemoradiation, values of this rate significantly decreased in contrast with GSH concentration after neoadjuvant chemotherapy in cases of stage IIB, regional metastases negative patients group, patients with a positive response to treatment, and patients who had no progression of the disease during the first 2 years after treatment. Statistically significant changes in GST during the treatment were not identified; the GST concentration after chemoradiation showed a statistically significant difference in GST concentrations in terms of the progression of the disease and disease without progression.

Conclusions: The results suggest that changes in the concentration of GSH during the treatment of locally advanced cervical cancer might be important for the prediction of the efficacy of the treatment. Statistically significant changes in GST concentration levels during the treatment were not observed.

* *Corresponding author at*: National Cancer Institute, Santariškių 1, 08660 Vilnius, Lithuania.
E-mail address: lina.daukantiene@vuoi.lt (L. Daukantienė).

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1. Introduction

Cervical cancer is the third most common cancer among women, and the fourth leading cause of death among women due to cancer worldwide [1]. It is the fourth most common female cancer, and is in seventh place according to the female death rate in Lithuania due to cancer; while among 15–44-year-old women, it is the most common cause of death from cancer [2,3].

The standard treatment for locally advanced cervical cancer is cisplatin-based chemoradiation [4]. However, the results of the treatment are not sufficient; the prognosis for a more advanced disease is still poor, with high rates of local and/or distant relapses. New and more effective treatment modalities are needed. Clinical trials are being carried out, and new chemotherapy regimens, chemotherapy and new combinations of target agents, and modifications to chemotherapy doses or schedules are being researched. Throughout these trials, new cytostatic combinations without cisplatin or new cisplatin-based combinations that are investigated as neoadjuvant or adjuvant therapies with radiation or chemoradiation are used. In order to individualize treatment, new prognostic and predictive factors are being searched for. A definite predictive significance is obtained by a series of already-known clinical, morphological and molecular factors in the case of cervical cancer: the stage, the size of the tumor, regional lymph nodes metastases, the histological type, the grade, lymph node and blood vessel invasion, and the blood hemoglobin level. However, even with identical data, the responses to the treatment and the survival rate of patients usually vary a lot. This encourages further scientific research in the field of molecular biology and genetics.

A significant role in cancerogenesis is played by changes in oxidative-reductive processes, which are reflected by the intensity of lipid peroxidation and the activity of antioxidative system enzymes [5–7].

Glutathione (GSH) is considered to be one of the main detox agents. It is known that the amount of GSH in the cell influences the sensitivity of cells to anticancer treatment and toxicity (a decrease in GSH increases drug toxicity). Therefore, the determination of the amount of GSH is crucial in order to foresee whether the cancerous cells will be sensitive to the effect of the drug, or if the effect of the drug toward normal cells will be innocuous. It is defined that, in comparison with a partial response, patients who suffer from cervical cancer have a significantly decreased amount of GSH in the blood and tumor, given a complete response to the treatment [8,9].

GSH changes can be affected by metabolism enzymes that use tripeptide as a substrate. In terms of cancer chemotherapy, sufficient activity of enzyme glutathione S-transferase (GST) is crucial, because during the enzyme catalyzed conjugation reacts with GSH, the solubility of drugs and other toxic materials in the water increases, and they are eliminated better; therefore, the effect on the organism is lower, which can cause a worse response to the treatment and a shorter survival [10].

The literature data shows that GSH and GST can be related to the response by cancer patients to the treatment and the survival rate. Approximate data from individual authors has described the significance of enzymes that participate in metabolic detoxification (GSH and GST) from a predictive aspect. The aim of our study was to determine changes in GSH and GST in the patients' blood serum, and assess their importance to the efficacy of treatment with applied intensive neoadjuvant chemotherapy based on a cisplatin and gemcitabine combination, followed by concurrent chemoradiation based on the same cytostatics combination for locally advanced stage IIB–IIIB cervical cancer.

2. Materials and methods

A total of 36 patients with untreated, histologically confirmed locally advanced FIGO stage IIB–IIIB cervical cancer were enrolled in the prospective phase II study in the Radiotherapy and Drug Therapy Center of Institute of Oncology, Vilnius University (now National Cancer Institute) between 2010 and 2012. Before the enrolment of patients, the Lithuanian Bioethics Committee and the State Medicine Control Agency approved the study. All the patients were informed, and written informed consent was obtained. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status 0–1; and adequate renal, liver and bone marrow functions. Patients were excluded if they were pregnant or breastfeeding, had a previous diagnosis of cancer, or had active cardiac disease. The median patients' age was 47.7 years, ranging from 29 to 67 years. The clinical and pathological characteristics of the patients are summarized in Table 1.

2.1. *Neoadjuvant chemotherapy*

Neoadjuvant chemotherapy (NACT) was administered weekly for 4 weeks, with a combination of cisplatin (30 mg/m^2) and gemcitabine (125 mg/m^2). Hydration, infusion duration and antiemetic prescription were administered according to standard guidelines. After a 14-day post-NACT period, a

Table 1 – Characteristics of the patients (n = 36).

Characteristic	n (%)
FIGO stage	
II B	17 (47)
III A	1 (3)
III B	18 (50)
Radiological assessment of the regional lymph nodes	
N0 (≤10 mm)	15 (42)
N1 (>10 mm)	21 (58)
Histopathology	
Squamous carcinoma	33 (92)
Adenocarcinoma	3 (8)
Grade	
G1	4 (11)
G2	6 (17)
G3	26 (72)
ECOG performance status	
ECOG = 0	32 (89)
ECOG = 1	4 (11)

detailed physical examination, including a pelvic examination, standard and perfusion computer tomography (CT) scan, were performed, to assess the response to the treatment. Images taken from CT with immobilization tools were used to plan external beam radiation (EBRT) and brachytherapy. A three-dimensional conformal EBRT dosimetry plan (3-D) was arranged.

2.2. Chemoradiation

Chemoradiation started in week 6. A combination of cisplatin (40 mg/m^2) and gemcitabine (125 mg/m^2) was administered weekly for 5 weeks during EBRT, beginning on the first day of radiation. The infusion of cytostatics was administered prior to radiation. EBRT using linear energy accelerator (15 MeV energy) to the whole pelvis was given to a total dose 50–50.4 Gy in 25–28 fractions over 5 weeks. Intracavitary brachytherapy was given following the completion of EBRT. Patients received 4 fractions (each 7 Gy) to point A high dose rate (HDR) 1–2 per week. The total dose delivered to point A was 89 Gy.

The response to the treatment was evaluated 2 months after the completion of the treatment. After that, patients were followed according to a standard monitoring program applied to patients treated for cervical cancer.

2.3. Assessment of treatment response and safety

The response to NACT was assessed by a pelvic examination, standard and perfusion CT scan, during which the size and structure of the cervix, parametrical invasion, and regional lymph nodes were assessed in the dynamics. A complete response (CR) is defined as the disappearance of all tumor signs, while a partial response (PR) to the treatment was a decrease in the tumor signs. Stable disease (SD) was diagnosed when the tumor signs remained unchanged. If the tumor size increased >20%, or a new lesion appeared, the diagnosis was given as progressive disease (PD).

The response to complete chemoradiation was assessed by a pelvic examination, cytological or histological examination (a biopsy must be carried out if a residual tumor is suspected), and CT scan. In order to exclude distant metastases, control radiological examinations of the chest, abdomen and pelvis were performed. CR was diagnosed if clinical and cytological tumor signs were not detected.

For safety evaluations, acute toxicity was assessed according to the Common Terminology Criteria for Adverse Events (National Cancer Institute CTCAE, version 3.0); and for acute and late radiotherapy toxicity, Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) Classification of adverse events was used.

2.4. Blood sample collection and analysis

Blood samples for GSH and GST were collected before the start of the treatment, 2 weeks after NACT (before the start of chemoradiation) and 2 months after the end of chemoradiation.

Samples for GSH and GST were stored at −70 °C until analysis. The levels of GSH and GST were evaluated by enzyme-linked immunosorbent assay ELISA (CUSABIO, BIOTECH, China), according to the manufacturer's guidelines. The absorbance of each well was detected with a microtiter reader (Shenzhen Mindray Bio-Medical Electronics Co, China) at a wavelength of 450 nm.

2.5. Statistical analysis

Non-parametric analysis was used, because the sample was not normally distributed. Pair-wise comparisons were conducted using the Wilcoxon signed rank test. The Mann–Whitney test was used to compare outcomes between groups. A statistical analysis was performed using the Statistical Package for the Social Sciences program (SPSS, 21.0). Frequencies and percentages were used for the categorical measures. The result was considered significant if a P value was <0.05.

3. Results

3.1. Treatment compliance and response to the treatment

According to the protocol, 97% of the patients completed all 4 planned cycles of NACT, while 28% of the patients completed all planned chemotherapy during EBRT. EBRT was fully carried out for 92% of the patients, and brachytherapy for 89%. The planned treatment was not fully realized because of toxicity, especially hematological toxicity. During NACT, 8% of patients had grade III neutropenia, and during the chemoradiation treatment grade III–IV leucopenia was experienced by 50% of the patients, neutropenia by 47%, thrombocytopenia by 19% of the patients, and grade III diarrhea by 8% of the patients.

3.2. Neoadjuvant chemotherapy

All 36 patients were evaluable for response. The proportion who had a response was 72% (n = 26; 95% CI, 64.76–79.69) at the end of NACT. All of these patients were diagnosed with PR. SD was observed in 28% (n = 10; 95% CI, 20.31–35.24) of patients. PD was not found in any patient at the end of NACT.

3.3. Chemoradiation therapy

In total, 34 patients were evaluable for response. Two patients did not come at the designated time for the assessment of the effect of the treatment. Clinical and cytological CR to treatment was observed in 94% (n = 32; 95% CI, 90.08–98.15) of patients (n = 32), and PD in 6% (n = 2; 95% CI, 1.85–9.92) of patients. One patient was diagnosed with lung and lymph node metastases, and another had bone metastases.

Further post-treatment observation of patients was assessed according to data of the progression – free survival (PFS) and data of overall survival (OS). At the time of interim data analysis (February 28, 2014), the mean follow-up time for all patients was 29.5 months (13.5–46.5 months). Data for 35 patients was assessed, because one of the patients was lost to the follow-up. As much as 71% (n = 25; 95% CI 63.79–79.06) of the patients were in continuous CR. PD was found in 29% (n = 10; 95% CI 20.94–36.21) of the patients. Currently, 77% (n = 27) of patients are alive, while 23% (n = 8) of patients are deceased.

3.4. Treatment toxicity

During NACT, grade III neutropenia was diagnosed for only 8% of the patients ($n = 3$), while 11% of the patients ($n = 4$) had grade III anemia, 50% ($n = 18$) grade III–IV leucopenia, and 47% ($n = 17$) grade III–IV neutropenia; 19% ($n = 7$) experienced grade III thrombocytopenia, and 8% of patients ($n = 3$) had grade III diarrhea during chemoradiation.

Among late toxicity were radiation cystitis, and radiation proctitis; nephrostomy emerged in two patients due to hydronephrosis caused by post-radiation fibrosis; two patients had surgery due to intestine obstruction.

3.5. Changes in GSH and GST during the treatment

After an analysis of the GSH and GST concentration of patients with stage IIB–IIIB cervical cancer, a statistically significant increase in GSH concentration levels after NACT was identified. Further chemoradiation for patients did not change the level of GSH. Statistically significant changes in GST during the treatment were not identified (Table 2).

After patients were grouped according to the FIGO stage of the disease, the GSH concentration after NACT increased significantly in both IIB and IIIB stages. After chemoradiation, values of this rate decreased in contrast with GSH concentration after NACT only in the case of stage IIB (Table 3). Statistically significant changes in GST concentration of stage IIB and stage IIIB cervical cancer were not identified during the treatment. It has to be noted that a comparison of GST data in patients with IIB and IIIB stages after chemoradiation showed a statistically significant difference.

After grouping patients according to regional lymph node metastases, which were defined using CT scan, the metastases negative regional lymph nodes (N0) patients group data showed a statistically significant decrease in GSH concentration after chemoradiation in contrast with GSH concentration after NACT; while the metastases positive (N1) group GSH concentration had a statistically significant increase after NACT, but stayed at the same level after chemoradiation (Table 4). Statistically significant changes in GST concentration in relation to the status of the lymph nodes during the treatment process were not observed.

In order to assess changes in GSH and GST concentrations according to the response to treatment by NACT based on a combination of cisplatin and gemcitabine, patients were divided into 2 groups: one of them ($n = 26$) had a positive (all of the patients partial) response to the treatment, while the other group ($n = 10$) had no response (stable disease). It has to be noted that, given the positive response toward the treatment, GSH concentrations showed a significant increase after NACT,

Table 2 – Changes in GST and GSH during treatment.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
GST, ng/mL	36	0.58 ± 1.11	36	0.76 ± 1.67	34	0.63 ± 0.90
GSH, μg/mL	36	23.51 ± 13.97*	36	35.29 ± 24.83*	34	32.01 ± 25.12

Values are mean ± standard deviation.
* $P < 0.05$.

Table 3 – GST and GSH changes during treatment according to the cancer stage.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
FIGO-II B						
GST, ng/mL	17	0.79 ± 1.41	17	0.78 ± 1.50	15	0.91 ± 1.08‡
GSH, μg/mL	17	23.51 ± 13.97*	17	37.84 ± 28.63*,†	15	31.60 ± 21.11†
FIGO-III B						
GST, ng/mL	19	0.40 ± 0.74	19	0.74 ± 1.85	19	0.39 ± 0.59‡
GSH, μg/mL	19	22.72 ± 13.88*	19	33.01 ± 21.42*	19	29.92 ± 27.43

Values are mean ± standard deviation. *, †, ‡$P < 0.05$.

Table 4 – GST and GSH changes during treatment according to regional lymph nodes metastases, defined by using radiological methods.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
N0						
GST, ng/mL	15	0.45 ± 0.37	15	0.38 ± 0.30	13	0.84 ± 1.19
GSH, μg/mL	15	26.70 ± 14.17	15	36.46 ± 28.51†	13	26.77 ± 21.43†
N1						
GST, ng/mL	21	0.68 ± 1.42	21	1.03 ± 2.15	21	0.48 ± 0.58
GSH, μg/mL	21	21.23 ± 13.72*	21	34.46 ± 22.54*	21	33.07 ± 26.44

Values are mean ± standard deviation. *, †$P < 0.05$.

Table 5 – GST and GSH changes during treatment according to the response to NACT.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
Positive response to NACT						
GST, ng/mL	26	0.48 ± 0.67	26	0.66 ± 1.58	26	0.56 ± 0.92
GSH, μg/mL	26	25.08 ± 14.83*	26	38.39 ± 23.60*†	26	33.21 ± 26.19†
No response to NACT						
GST, ng/mL	10	0.85 ± 1.85	10	1.01 ± 1.95	10	0.77 ± 0.78
GSH, μg/mL	10	19.41 ± 11.05	10	27.24 ± 27.41	10	24.55 ± 19.74

Values are mean ± standard deviation. *, †$P < 0.05$.

Table 6 – GST and GSH changes during treatment according to a complete response to chemoradiation.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
GST, ng/mL	32	0.62 ± 1.19	32	0.56 ± 1.15	32	0.63 ± 0.90
GSH, μg/mL	32	23.42 ± 14.38*	32	35.62 ± 25.01*†	32	32.01 ± 25.12†

Values are mean ± standard deviation. *, †$P < 0.05$.

Table 7 – GST and GSH changes in the blood, after the treatment of cervical cancer according to the disease progression during the first 2 years after the treatment.

Factor	N	Before treatment	N	After NACT	N	After chemoradiation
Progression-free						
GST, ng/mL	25	0.39 ± 0.31	25	0.37 ± 0.35	25	0.74 ± 1.00‡
GSH, μg/mL	25	24.20 ± 15.44*	25	39.07 ± 27.02*†	25	34.62 ± 27.15†
Disease progression						
GST, ng/mL	10	1.10 ± 2.03	10	1.77 ± 2.99	10	0.33 ± 0.32‡
GSH, μg/mL	10	21.70 ± 9.61	10	25.46 ± 14.90	10	21.17 ± 13.22

Values are mean ± standard deviation. *, †, ‡$P < 0.05$.

and a statistically significant decrease after chemoradiation, in contrast to its level after NACT. Patients who had no response toward NACT showed no significant changes in GST and GSH concentrations during the process of the treatment (Table 5).

After finishing the complete chemoradiation defined by the protocol, calculations revealed that 2 patients did not come to the assessment of the effects of the treatment, 2 were identified with a progression of the disease, and 32 patients had a complete response. GST and GSH changes during the treatment process were assessed, and the results showed that the GSH concentrations of patients with CR underwent a statistical increase after NACT, and a decrease after chemoradiation (Table 6).

Further patient observation after the treatment was assessed according to the patient survival rate without progression of the disease. During the first 2 years since the beginning of the treatment, 29% ($n = 10$) of patients encountered a progression of the disease, and 2 of them were identified with a progression of the disease immediately after chemoradiation. Data relating to changes in GST and GSH concentrations according to the progression of cervical cancer during the first 2 years since the beginning of the trial is presented in Table 7. Patients who did not have a progression in the disease were identified with a statistically significant increase in GSH concentration after NACT, and a statistically significant decrease after chemoradiation. Patients who had a progression of the disease during the first 2 years after the treatment showed no statistically significant changes in GSH concentration during the process of the treatment. GST concentration changes during the course of the treatment were not identified, but the GST concentration after chemoradiation showed a statistically significant difference in GST concentrations in terms of the progression of the disease and the disease without progression.

4. Discussion

On one hand, free radicals are essential to most of the cell process regulation; on the other hand, high amounts of free radicals, especially active oxygen forms, tend to indicate oxidative stress. The antioxidative system prevents oxidative stress damage to the organism. Changes in oxidative stress have been investigated intensively in various cancers, including cervical cancer. But there is a lack of data that reflects the importance of oxidative stress in terms of predictive and prognostic aspects.

Mukundan et al. [11] examined the concentration of GSH and glutathione-peroxidase (GSH-Px) in patients with cervical cancer pre- and post-radiation, and a control group of healthy women. The results revealed that the levels of GSH and GSH-Px in patients with cervical cancer were significantly lower than those of the control group. This study also demonstrated the effect of radiation therapy on the antioxidant system, but a relationship between GSH, GSH-Px changes

and the response to treatment, in contrast to our study results, was not observed. Unlike the results of Bhuvarahamurty et al. [12], GSH and GST values after the treatment did not reach the values of the control group.

Sharma et al. [13] investigated the blood lipid peroxide, GSH, GSH-Px, GST, catalase (CAT) and superoxide dismutase (SOD) concentration levels in 60 patients with cervical cancer before neoadjuvant chemotherapy, 2 weeks after chemotherapy, and 2 weeks after radiation, and then compared the results with data from a control group of 60 healthy women. Before the treatment, in the blood of patients with cervical cancer, the lipid peroxide level was significantly higher; while the GSH, GSH-Px, GST, SOD and CAT were significantly lower, in contrast with the lipid peroxide levels and enzyme activity in the blood of the control group. After chemotherapy, the level of lipid peroxide decreased significantly; while the GSH, GSH-Px, GST, SOD and CAT levels slightly increased. After radiation, the mentioned levels returned to a normal state ($P < 0.01$). According to researchers, the normalization of lipid peroxide levels and antioxidative system enzymes could be beneficial in terms of response assessment. In our study a statistically significant increase in GSH concentration levels after NACT was identified. Further chemoradiation for patients did not change the level of GSH. Statistically significant changes in GST concentration levels during the treatment were not identified.

Wozniak et al. [14] investigated CAT and GSH-Px activity in patients with cervical cancer. Patients were divided into 3 groups, based on treatment options: patients who had brachytherapy before surgery; patients who had brachytherapy after chemoradiation; and patients who had external treatment after brachytherapy. CAT activity increased after the treatment, while GSH-Px decreased, in contrast with the activity of these enzymes in the control group; however, they did not reach the levels of examined enzymes determined in the control group. CAT and GSH-Px levels returned to a normal value 6 months after the treatment.

Mila-Kierzenkowska et al. [15] examined changes in CAT, GSH-Px, thiobarbituric acid reactive substances (TBARS) of plasma and erythrocytes among 84 patients with cervical cancer treated with brachytherapy, and compared the results with a control group of 30 healthy women. Blood samples were collected before radiotherapy, the day after each brachytherapy procedure, and 6 months after the end of the treatment. During the treatment, no significant differences in the response to the treatment and activity of antioxidant enzymes or lipid peroxide product levels changes were observed. However, 6 months after the treatment, CAT, GSH-Px and TBARS concentrations reached the initial pre-treatment level, which, according to the authors, shows a positive response toward the treatment.

Demirici et al. [16] examined GSH, GSH-Px and SOD concentrations and the malondialdehyde (MDA) level before and after (chemo)radiotherapy in 35 patients, and compared the results to data from a control group of 35 healthy women. Antioxidant levels differed significantly between patients and healthy women. The examination of antioxidant levels in patients with cervical cancer before and after radiation did not show any significant difference. However, an analysis of the amount of GSH after radiation revealed that patients who did not show a response to radiation had a significantly increased amount of GSH, in contrast with those who showed a response to radiation ($P < 0.01$). Similar results were achieved in our study: the GSH concentrations of patients with CR underwent a significant decrease after chemoradiation. Therefore, it is possible that GSH may be a predictive factor for the treatment response of patients with cervical cancer.

The predictive role of GSH for determining the response to radiotherapy was also investigated by Jadhav et al. [17]. Blood and tissue samples were collected from 45 cervical cancer patients before and after the first fraction of radiation. The levels of GSH showed a significant decrease in blood and tissue after the first fraction; moreover, a correlation between the response to treatment and GSH levels was determined. All the patients who received CR were noticed with a more than 70% decrease in GSH concentration in the blood and tumor. Patients who were observed to have a tumor reduction of less than 50% (stable disease) had GSH concentration decreases of less than 50%. Those who were diagnosed with PR prior to the treatment had GSH concentration decreases to approximately 50%–70% in the blood serum and tumor tissue. In our study, patients who showed a response to NACT and chemoradiation were observed with a statistically significant GSH decrease in the blood serum after chemoradiation. Patients whose response was not observed did not reveal any statistically significant changes in GSH concentration.

Most authors' in vitro and in vivo studies and their results show that GST plays an important role in the formation of tumor cell resistance to chemotherapy. It was established that increased GST expression in tumor cells determines resistance to cisplatin and other platinum-compounds [18–20]. However, there is evidence in several trials showing no correlation between GST levels in tumor cells and drug resistance to chemotherapy [21]. In addition, it has been determined that the GST level in serum or plasma can be used as a marker which allows us to predict the response to chemotherapy [22,23]. This data reveals the importance of further investigations in order to assess the role of GST in the formation of drug resistance to chemotherapy, because it could be one of the biomarkers that help to create a more effective strategy for significant GSH concentration changes during treatment.

According to the data of the study results of Vidyasagar et al. [24], a serum GSH decrease can be a predictive factor in the treatment response for cervical cancer patients treated with radiation. The data shows that the GSH concentration decreases significantly after chemoradiation, especially for patients who reach CR, in contrast with those who do not receive any response at all. Our study demonstrated the same results.

Sharma et al. [25] examined 90 patients with locally advanced cervical cancer, and a control group of 90 healthy women. Blood samples were collected and analyzed before and after NACT, after radiotherapy, and a year after the treatment. Before the treatment, the level of plasma lipid peroxide was higher, while the level of endogenous antioxidants and antioxidant enzymes, including GSH and GST, was lower, in contrast with levels of lipid peroxide and antioxidants and antioxidant enzymes in the control group. After chemotherapy, the level of lipid peroxide decreased significantly in patients who had CR prior to treatment, in contrast

with those who had PR or no response at all. These differences remained significant after the treatment and during the follow-up period. After chemotherapy, antioxidant enzymes increased ($P < 0.05$) among patients with CR, in contrast with patients with PR or no response at all. This proves that the investigated parameters can have a predictive role in the treatment response to chemotherapy and radiation. Our study found statistically significant increase in GSH levels after neoadjuvant chemotherapy, and these levels remained unchanged after the end of chemoradiotherapy. We observed decline in GSH levels after chemoradiotherapy compared to GSH levels after chemotherapy in patients with good response to treatment. We did not observe any changes in GST levels in this study.

Prabhu et al. [26] measured levels of serum GST before and after radiation. The study results showed that alterations in serum GST levels may help to predict the response to radiation. The data from our research shows that statistically significant changes in GST concentrations during treatment were not observed. However, a statistically significant difference was determined among patients who were compared according to the time of the progression of the disease after chemoradiation.

5. Conclusions

Based on the data from our study, it can be stated that changes in GSH concentration in the process of the treatment of locally advanced cervical cancer might be significant to the response to the treatment. Statistically significant changes in GSH concentration levels during the process of the treatment in the patient group with a positive response and no established disease progression during 2 years after the treatment, in contrast with the absence of concentration changes in the blood serum of patients with no response to the treatment or ones with an established relapse after the treatment, points to GSH as an important predictive factor. Statistically significant changes in GST concentration levels during the treatment were not observed.

Conflict of interest

The authors state no conflict of interest.

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