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Dynamics of certain parameters indicating antioxidative system status in surgically treated gastric and colorectal cancer patients

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Background. Amounts of total glutathione (GSH), malondialdehyde (MDA), catalytic activities of catalase (CAT) and glutathione S-transferase (GST) in serum of gastric and colorectal cancer patients were investigated to determine the dynamics of antioxidative system status under surgical treatment.

Materials and Methods. 37 gastric and 51 colorectal cancer patients of early (I, II) and advanced (III, IV) stages treated by surgery were included. MDA was tested by thiobarbituric acid (TBA) assay. GSH was determined using recycling system by 5,5-ditiobis(2-nitrobenzoic) acid (DTNB) known as Ellman's reagent (DTNB) and glutathione reductase (GR). CAT was defined under formation of a hydrogen peroxide/ammonium molybdate complex. GST was measured by formation of GSH/1-chloro-2, 4-dinitrobenzene (CDNB) conjugate.

Results. MDA and GSH amounts decreased, and GST activity increased in 1 week and 2 weeks after the surgical treatment of both gastric and colorectal cancer patients if compared with the pre-surgical period. MDA level and CAT activity were also diminished comparing the indices of 1 week and 2 weeks post-surgical period. CAT activity decreased only in the post-surgical period of 2 weeks for gastric cancer patients while the parameter dropped for both 1 week and 2 weeks period colorectal cancer patients. A lower GSH amount was detected for advanced stages (III + IV) gastric cancer patients 1 week after the surgery. GST activity was higher for early stages (I + II) only prior to the treatment. No significant differences among the stages were determined for colorectal cancer patients as well as between two age groups (20–59 and 60 + years old) for both cancer localizations.

Conclusions. The duration of post-surgical period affects antioxidative system parameters. No reliable differences were determined for most parameters pairs comparing early to advanced cancer stages. The age was shown to be not essential reason of dynamics of the parameters and the process was rather dependent on the tumour localization.

Key words: surgery, gastric cancer, colorectal cancer, antioxidative system

INTRODUCTION

Oxidative stress as a prolonged and abnormal excess of reactive oxygen species (ROS) is one of the main factors of cancer risk. Results of direct measurements of ROS in cells and also determination of antioxidative system status parameters both in cells and in body fluids of cancer patients are still diverse, indicating many factors to have effect on the dynamics of the parameters. Oxidative stress could be also stimulated by tumour progression and even by cancer treatment (1–3). Surgical intervention induces additional stress also affecting the dynamics of antioxidative system parameters (4).

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Colorectal and gastric cancers are among common tomours worldwide (5). These cancer localizations were also found to be among 10 most prevalent cancer localizations in Lithuania in 2005. Unfortunately, these cancers are rather often diagnosed at advanced stages, and patients usually undergo surgical treatment of large extent (6).

Dynamics of lipid peroxidation indices, of certain antioxidant compound levels and of antioxidative enzyme activities in post-surgical period of cancer patients could indicate successful treatment or possible forthcoming complications as well as to serve as prognostic factors (7, 8). Malondialdehyde (MDA) level is still the main sensitive parameter of lipid peroxidation (9). Total glutathione (GSH) amount is an important antioxidant index as antioxidant defence is regulated by redox pair of reduced and oxidezed glutathione (GSH/GSSG). Catalase (CAT) activity indicates a primary antioxidative action, while glutathione S-transferase (GST) activity shows the detoxication function of GSH (10, 11).

The goal of the present research was to investigate the four selected parameters in serum of gastric and colorectal cancer patients of early (I, II) and advanced (III, IV) stages treated by surgery in order to determine the dynamics of antioxidative system status in the post-surgical period.

MATERIALS AND METHODS

Patients. 37 gastric and 51 colorectal cancer patients of early (I, II) and advanced (III, IV) stages treated by surgery were included in the study following the criteria of age (over 18) and Hb prior to the treatment (exceeding 110 g/l). Patients were grouped considering cancer stage and age as summarized in Tables 1 and 2.

Table 1. **Groups of gastric and colorectal cancer patients considering stage of the disease**

Tumour localization	N	Stages			
		I	II	III	IV
Gastric	37	12	9	10	6
Colorectal	51	5	17	24	5

Table 2. **Groups of gastric and colorectal cancer patients considering age**

Tumour localization	N	Age, years			
		20–34	35–49	50–64	65 and over
Gastric	37	1	6	12	18
Colorectal	51	0	4	22	25

3 samples of blood serum of each patient were tested: prior to the treatment, 1 week and 2 weeks after the surgery.

Methods. The status of antioxidative system was evaluated by determining lipid peroxidation product MDA, antioxidant GSH and enzymes CAT and GST. All the parameters were tested spectrophotometrically. MDA level was determined by TBA assay based on the release of color MDA/TBA complexes as described in (12). CAT activity was measured as the development of color under the formation of a hydrogen peroxide / ammonium molybdate complex (13). GSH amount was detected using a recycling system by DTNB and GR following the method of (14). GST activity was measured as the rate of GSH / CDNB conjugate formation as described in (15). Dynamics of MDA (nmol/ml), CAT (nmol/l/min), GSH (mkmol/ml) and GST (nmol/ml/min) indices was evaluated comparing values in prior-to and post-surgical periods.

Statistical analysis of the data was performed by Paired Samples Test and Independent Samples T-test using program SPSS 14.0. Differences were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Although gastric and colorectal cancers are among the most common localizations in Lithuania (6) the treatment effect is not always efficient. That is particularly apparent in cases when cancer is diagnosed in advanced stages. Search of cancer biomarkers at various biological levels is still crucial to provide correction of the treatment tactics for individual patients when analysing the dynamics of those indices.

Parameters of antioxidative system status are promising in search for new biomarkers indicating the prognosis of treatment and the course of the disease (16). The four selected indices were examined in the present study for gastric and colorectal cancer patients. The mean of each parameter was compared in the following manner: 1) pre-surgery with 1 week post-surgery; 2) pre-surgery with 2 weeks post-surgery; 3) post-surgical periods of 1 week and 2 weeks. Neither cancer stage nor patients age were taken into consideration in this comparison. Paired Samples Test was used for data analysis. Only statistically reliable variants or those having tendency to reliability are discussed. Results are presented in Table 3.

Table 3. **Comparison of malondialdehyde (MDA, nmol/ml), catalase (CAT, nmol/l/min), total glutathione (GSH, mkmol/ml) and glutathione S-transferase (GST, nmol/ml/min) indices of cancer patients within pre-surgery (1) and post-surgical period of 1 week and 2 weeks (2 and 3, respectively)**

Pairs	Mean Difference	
	Gastric cancer	Colorectal cancer
MDA1 / MDA2	↓ 3.776 ± 0.673*	↓ 2.983 ± 0.603*
MDA1 / MDA3	↓ 6.175 ± 0.621*	↓ 5.133 ± 0.686*
MDA2 / MDA3	↓ 2.197 ± 0.622*	↓ 2.019 ± 0.504*
CAT1 / CAT2	not sufficient	↓ 4.406 ± 1.757*
CAT1 / CAT3	↓ 4.739 ± 1.320*	↓ 8.115 ± 1.404*
CAT2 / CAT3	↓ 3.720 ± 1.154*	↓ 4.104 ± 1.139*
GSH1 / GSH2	↓ 0.062 ± 0.014*	↓ 0.057 ± 0.009*
GSH1 / GSH3	↓ 0.040 ± 0.011*	↓ 0.040 ± 0.010*
GSH2 / GSH3	↑ 0.022 ± 0.012**	↑ 0.018 ± 0.010**

* $p < 0.05$ – statistically reliable

** $0.05 < p < 0.1$ – tendency to statistical reliability

↓ – reduced; ↑ – increased

It is evident that the MDA level decreased statistically reliably in all the pairs analysed both for gastric and colorectal cancer patients. CAT activity also decreased in most pairs with the exception of pair CAT1 / CAT2 for gactric cancer patients. The significant drop of GSH was determined in 1 week and 2 weeks after surgical treatment if compared with the pre-surgical period, however, only a tendency towards reliable difference of the parameter was found while comparing values within the post-surgical period of 1 week and 2 weeks for both tumour localizations (Table 3). No reliable dynamics of GST was determined.

The role of cancer stage for alterations of MDA, CAT, GSH and GST parameters was evaluated. In subject, patients were divided to two groups of early (I, II) and advanced (III, IV) cancer stages. Parameters between those groups were compared considering pre- and post-surgical periods as noted above.

A statistically reliable lower amount of GSH (↓ 0.083 ± 0.302) was determined to be one week after surgery for advanced stages gastric cancer patients if compared with the early stages. Moreover, GST activity was significantly lower prior to surgery (↓ 185.788 ± 77.124) and showed a tendency to decrease 2 weeks after surgery (↓ 172.824 ± 99.715) for advanced stages gastric cancer patients. No statistically reliable differences of any parameters were determined for colorectal cancer patients while comparing among cancer stages. Further experiments should be performed to evaluate the dynamics of antioxidative system parameters as the antioxidant status of colon and gastric

cancer patients was noticed to change with the disease progression (9, 17).

The role of the patients age for dynamics of all the parameters investigated was also evaluated. Patients were compared between 2 age groups only (20–59 and 60+) taking into account the incomplete number of patients in age groups 20–34 and 35–49 (Table 2). Parameters between those groups were compared considering pre- and post-surgical period as noted above. No statistically reliable differences of parameters between the two age groups were determined either within the pre-surgery period or during the post-surgical one for both cancer localizations.

It could be suggested that the drop of MDA level within the post-surgical period if compared with the pre-surgical time for both gastric and colorectal cancer patients shows the decrease of oxidative stress as part of the weakened lipid peroxidation. Even post-surgical period of one week showed a lower amount of MDA that could indirectly mean a possible effectiveness of surgical treatment. It is necessary to investigate the relation of the parameter dynamics with the changes of biomarkers of clinical order to provide prognosis of surgical treatment (18). The lower amount of GSH after surgery should illustrate a possible result of operative stress rather than the reduced function of antioxidative system since GSH is crucial as a modulator of immune system and detoxication as well (19). GSH level increased within the post-surgical period of 2 weeks but it is not statistically reliable.

GST activity shows the detoxication function of GSH. The increased catalytic activity prior to the surgical treatment and the decreased parameter after the treatment were found to be insufficient. Most probably, alteration of GST activity should be related to changes of GSH as the enzyme substrate. On the other hand, detoxication process is specific in the case of cancer. Consequently, complex investigations combining experiments on enzymatic and molecular levels are necessary to evaluate the function of GST during cancer progression and surgical treatment. It was already shown that GST genetical polymorphism is related to cancer risk (20), so GST is a candidate as a cancer biomarker.

Very few sufficient differences of parameters comparing early and advanced cancer stages could not allow adequate summarizing of the results, although it looks as if antioxidative system status is better at initial stages as the data at least for gastric cancer patients demonstrated. Besides, further measurements are required to evaluate the role of patients' age to antioxidative system status showing statistically reliable differences of the parameters.

Comparison of the data of the present study with the results of other authors is complicated since a group of cancer patients is most often compared with a control group of healthy volunteers. Also it should be noted that difficulties of comparison appear as not only parameters but also methods of their determination as well as cancer localization and method of treatment must be the same.

CONCLUSIONS

MDA and GSH amounts and CAT activity decreased and GST activity increased statistically reliably in 1 week and 2 weeks after the surgical treatment for both gasric and colorectal cancer patients in comparison with the pre-surgical period. MDA level and CAT activity were also diminished sufficiently while comparing the values of indices within the post-surgical period of 1 week and 2 weeks.

Lower GSH amount one week after surgery was detected for advanced stages (III + IV) than that for early stages (I + II) gastric cancer patients. GST activity was higher for early stages gastric cancer patients only prior to surgical treatment. No statistically reliable differences among cancer stages were determined for colorectal cancer patients. Finally, no sufficient differences were found for patients with gastric and colorectal cancer within the post-operational period of 1 week and 2 weeks in comparison with the pre-surgical one as well as in comparing indices of 1 week and 2 weeks post-surgical period in two age groups (20–59 and 60+).

To sum up, 1) the duration of post-surgical period affects antioxidative system parameters; 2) no reliable differences were determined for most parameters comparing the early cancer stages with the advanced ones; 3) the age was shown not to cause essential dynamics of parameters but the process was dependent on the tumour localization.

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SKRANDŽIO BEI STOROSIOS ŽARNOS VĖŽIU SERGANČIŲ IR CHIRURGINIU BŪDU GYDYTŲ LIGONIŲ ANTIOKSIDACINĖS SISTEMOS RODIKLIŲ POKYČIAI

S a n t r a u k a

Tikslas. Tyrimo tikslas buvo nustatyti bendro glutationo (GSH) ir malondialdehido (MDA) kiekių, katalazės (CAT) bei glutationo S-transferazės (GST) katalitinių aktyvumų pokyčius kraujo serume, rodančius antioksidacinės sistemos būklę, skrandžio bei storosios žarnos vėžiu sergantiems ligoniams, kuriems buvo taikytas chirurginis gydymas.

Pacientai ir metodai. Tiriamųjų grupes sudarė 37 skrandžio ir 51 storosios žarnos ankstyvųjų (I, II) bei vėlyvųjų (III, IV) stadijų vėžiu sergantys ir chirurginiu būdu gydyti ligoniai. MDA kiekis nustatytas tiobarbitūrinės rūgšties (TBA) testu, GSH kiekis – dviguba reakcija su Ellmano reagentu (DTNB) ir glutationo reduktaze (GR), CAT fermentinis aktyvumas nustatytas sekant vandenilio peroksido ir amonio molibdato komplekso susidarymą, GST katalitinis aktyvumas – pagal GSH/1-chloro-2, 4- dinitrobenzeno (CDNB) konjugato susidarymą. Visi rodikliai nustatyti spektrofotometriniu metodu.

Rezultatai. Sergančiųjų ir skrandžio, ir storosios žarnos vėžiu MDA ir GSH kiekiai sumažėjo, o GST aktyvumas padidėjo pirmą ir antrą savaitę po operacijos, lyginant su priešoperaciniu periodu. MDA kiekis bei CAT aktyvumas taip pat sumažėjo, palyginus vienos savaitės pooperacinį periodą su dviejų savaičių periodu. CAT aktyvumas skrandžio vėžiu sergančių ligonių sumažėjo tik praėjus dviem savaitėms po chirurginio gydymo, tuo tarpu sergančių storosios žarnos vėžiu šis rodiklis sumažėjo tiek po vienos, tiek ir po dviejų savaičių. Vėlyvųjų stadijų (III + IV) skrandžio vėžiu sergantiems ligoniams pirmą savaitę po operacijos nustatytas mažesnis GSH kiekis. GST aktyvumas prieš operaciją buvo didesnis ankstyvųjų vėžio stadijų (I + II) ligonių. Lyginant tiriamus rodiklius tarp įvairių stadijų storosios žarnos vėžiu sergančių ligonių, statistiškai reikšmingų skirtumų nenustatyta. Nerasta reikšmingų rodiklių pokyčių ir lyginant sergančiuosius abiejų lokalizacijų vėžiu pagal amžių (20–59 m., 60 m. ir daugiau).

Išvados. Pooperacinio periodo trukmė turi įtakos antioksidacinės sistemos rodiklių kaitai. Lyginant tirtų rodiklių reikšmes prieš ir po operacijos, nustatyti tik dviejų iš jų (GSH kiekio ir GST aktyvumo) reikšmingi skirtumai tarp vėžio stadijų. Ligonių amžius neturėjo ypatingos reikšmės tirtų rodiklių pokyčiams, tuo tarpu vėžio lokalizacija buvo svarbi.

Raktažodžiai: chirurginis gydymas, skrandžio vėžys, storosios žarnos vėžys, antioksidacinė sistema