Turk J Gastroenterol *2016; 27: 336-41*

Glutathione and glutathione S-transferase levels in patients with liver metastases of colorectal cancer and other hepatic disorders

LIVER

Edita Baltruskeviciene[1], Birutė Kazbariene[2], Robertas Badaras[3], Loreta Bagdonaitė[4], Aurelija Krikštaponienė[2], Linas Zdanavičius[5], Eduardas Aleknavicius[1,6], Janina Didziapetrienė[6,7]

[1]Center of Radiation and Medical Oncology, National Cancer Institute, Vilnius, Lithuania
[2]Laboratory of Cancerogenesis and Cancer Epidemiology, National Cancer Institute, Vilnius, Lithuania
[3]Clinic of Anaesthesiology and Intensive Care, Vilnius University Faculty of Medicine, Vilnius, Lithuania
[4]Department of Physiology, Biochemestry, Microbiology and Laboratory Medicine, Vilnius University Faculty of Medicine, Vilnius, Lithuania
[5]Republican Vilnius University Hospital, Vilnius, Lithuania
[6]Clinic of internal Diseases, Family Medicine and Oncology, Vilnius University Faculty of Medicine, Vilnius, Lithuania
[7]Center of Research, National Cancer Institute, Vilnius, Lithuania

Original Article

ABSTRACT

Background/Aims: Glutathione and glutathione S-transferases (GST) are involved in cell defence against reactive oxygen species, which induces oxidative stress and are associated with different chronic diseases. The aim of the present study was to determine the differences in reduced glutathione (GSH) and GST levels in patients with different liver diseases.

Materials and Methods: Overall, 114 patients were enrolled in this study: 58 patients with colorectal cancer (18 without and 40 with liver metastases), 27 with liver steatosis, 29 with alcoholic cirrhosis and a group of 40 healthy volunteers. The levels of GSH and GST in blood serum were evaluated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's guidelines.

Results: Significant differences in GSH and GST levels were observed in most of the groups compared to the healthy volunteers (GSH: 52.72 μg/mL, GST: 0.53 ng/mL): with hepatic steatosis (GSH: 17.04 μg/mL, $p<0.001$; GST: 5.89 ng/mL, $p<0.001$), alcoholic cirrhosis (GSH: 62.04 μg/mL, $p<0.003$; GST: 0.94 ng/mL, $p<0.001$) and liver metastases (GSH: 37.84 μg/mL, $p<0.001$, GST: 1.25 ng/mL, $p=0.747$).

Conclusion: The different GSH and GST levels in patients with colorectal cancer liver metastases, liver steatosis and alcoholic cirrhosis indicate the differences in antioxidative system damage and its compensatory possibilities and could serve as potential biomarkers for its correction.

Keywords: Glutathione, glutathione-S-transferase, colorectal cancer, liver steatosis, liver cirrhosis

INTRODUCTION

The formation of reactive oxygen species (ROS) is a normal consequence of essential biochemical processes. However, the excessive formation of ROS and their reactive derivatives induces oxidative stress, which is associated with different chronic pathologic conditions, including cancer as well as liver diseases (1-5).

There are special enzymatic and non-enzymatic systems protecting cells from the destructive effects of ROS. Those systems involve primary antioxidative enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)] secondary antioxidative enzymes [glutathione reductase (GR), glutathione-S-transferase (GST), glucose 6-phosphate dehydrogenase (G6PDH)] and molecules such as glutathione, heat shock proteins, vitamins A, C, E and others.

Glutathione and glutathione-dependent enzymes, such as GSTs, are involved in cell defence against ROS. The glutathione system participates not only in the antioxidant defence system but also plays an important role in many processes on a molecular, cellular and organism level; therefore, disturbances in glutathione

Address for Correspondence: Edita Baltruskeviciene E-mail: edita.baltruskeviciene@nvi.lt
Received: November 19, 2015 **Accepted:** April 7, 2016

system homeostasis are involved in pathogenesis and the progression of cancer and liver diseases (6-8). GSTs are a family of detoxification enzymes that catalyse the conjugation of reduced glutathione (GSH) to a wide variety of endogenous and exogenous compounds, making them less biologically active, more water soluble and more quickly eliminated from an organism. Hence, GSTs are important in controlling toxic products by generating lipid oxidation and oxidative stress. Some members of GSTs, such as π (pi) and μ (mu) classes of cytosolic GSTs, play a regulatory role in the mitogen activated protein (MAP) kinase pathway that participates in cellular survival and death signalling (7,9).

It has been shown that oxidative stress plays a crucial role in tumour carcinogenesis. ROS and their reactive derivatives cause deoxyribonucleic acid (DNA) damage, interact with oncogenes and tumour suppressor genes and influence the tumour microenvironment by inducing inflammation/repair and angiogenesis. An increased level of oxidative stress and redox misbalance are very important in colorectal cancer and tend to be associated with different stages of disease, its progression and responsiveness to treatment and treatment toxicity (10,11). The liver represents the main organ subjected to metastases from colorectal cancer. The formation of hepatic metastases is a multistep process of cancer cells spreading to the liver and involves the generation of new blood and lymph vessels, growth, local invasion with a breakdown and cross-talk of the host matrix, adhesive alteration, transport to other sites and survival in a new environment.

Liver steatosis and cirrhosis are liver diseases frequently associated with the toxic effects of alcohol, high fat diets and certain drugs. Liver steatosis is a reversible condition of triacidglycerides accumulation in hepatocytes, which could be accompanied by inflammation, and is also called steatohepatitis. *Cirrhosis* is fibrosis of the liver tissue and is seen at the terminal stages of chronic liver diseases and leads to liver dysfunction. In the case of these diseases, oxidative processes generate the formation of ROS. They are highly reactive molecules. Their direct effects on cellular proteins, DNA and lipids can cause different oxidative modifications and lead to disruption of their normal physiological functions and can contribute to the progression of these diseases (1,2).

The literature data concerning the antioxidant defence systems' capacity in the case of chronic liver diseases are controversial. The changes in the activity of antioxidant enzymes, such as SOD, CAT and GST-Px, were examined in children with liver diseases. Chrobot et al. (12) observed a statistically significant decrease of CAT and SOD activities and a decrease in GSH-Px activity in children with chronic hepatitis B and C. In the case of infants with cholestatic liver disease due to bile duct damage and children with portal vein thrombosis, the SOD activity was increased (13). A decreased GSH level in chronic liver diseases has been reported in many reports (6,14). GST is a sensitive marker in the diagnosis of alcoholic liver disease (15) as well a reliable marker in monitoring the response to chronic liver disease treatment (16).

According to Inokuma et al. (10), ROS are associated with increased tumour invasion but not with liver metastases of the colorectal cancer. On the other hand, Scibior et al. (17) reported that liver metastases may influence changes in the antioxidant system.

The aim of the present study was to evaluate the alterations in GSH and GST in patients with or without liver metastases of colorectal cancer and to compare these alterations in patients with liver steatosis or alcoholic cirrhosis and healthy volunteers.

The novelty of this study is the comparison of the investigated parameters of glutathione system function in different hepatic pathologies (liver metastases of colorectal cancer, liver steatosis and alcoholic cirrhosis).

MATERIALS AND METHODS

Study and control groups

114 patients were enrolled in this study (Table 1).

18 colorectal cancer patients without liver metastases: stage III adenocarcinomas of the colon (100%), grade 2 (83%) and grade 3 (17%).

40 colorectal patients with liver metastases: stage IV adenocarcinomas of the colon (60%) and rectum (40%), grade 1 (2%), grade 2 (90%) and grade 3 (8%).

Table 1. Study group characteristics

Groups	Number of patients (%)	Age	Male n (%)	Female n (%)
Colorectal cancer with liver metastases	40 (26.0)	52.50 (±15.62)	21 (52.5)	19 (47.5)
Colorectal cancer without liver metastases	18 (11.7)	57.66 (±9.43)	6 (33.3)	12 (66.6)
Steatosis	27 (17.5)	43.7 (±12.81)	19 (70.4)	8 (29.6)
Alcoholic cirrhosis	29 (18.8)	51.48 (±11.46)	23 (79.3)	6 (20.7)
Healthy volunteers	40 (26.0)	41.0 (±9.3)	9 (22.5)	31 (77.5)
Total	154 (100.0)		78 (50.6)	76 (49.4)

27 patients with steatosis and admitted to the hospital due to liver function disturbances.

29 patients with alcoholic cirrhosis and admitted to the hospital due to bleeding from varicose veins, exhaustion, malnutrition, infection complications, decompensated ascites, haemodynamic disorder and with a history of alcohol consumption less than 5 years.

40 healthy volunteers; healthy persons with no history of any liver diseases in the past.

All the pathologies were diagnosed by clinical, radiological and histopathological examinations. For the study groups, blood samples were collected before the systemic treatment; for colorectal cancer patients, it was collected 1 month after the surgery for the primary tumour.

The study was approved by the Regional Biomedical Research Ethics Committee and conducted in accordance with the Helsinki declaration. All the patients signed written informed consent before entering the study.

Blood sample collection and analysis

Blood samples were collected into a tube without additives, coagulated naturally at room temperature for 10–20 min and then centrifuged for 20 min at 3000 r/min. The supernatant was collected and stored at –70°C until the analyses. The levels of GSH and GST were evaluated in blood serum by enzyme-linked immunosorbent assay (ELISA) (Human Glutathione ELISA Kit and Human Glutathione S-Transferases ELISA Kit; CUSABIO, Wuhan, China) according to the manufacturer's guidelines. The absorbance of each well was detected with a micro-plate reader (MR-96A; Mindray Bio-Medical Electronics Co.; Shenzen, China) at a wave length of 450 nm. Sensitivity of assays: the minimum detectable dose of human GST is typically less than 0.39 ng/mL, whereas that for GSH is typically less than 0.195 µg/mL. The sensitivity of this assays, or the Lower Limit of Detection, was defined as the lowest protein concentration that could be differentiated from zero. These assays have a high sensitivity and specificity for the detection of human GST and GSH. No significant cross-reactivity or interference between human GST or GSH and the analogues were observed.

Statistical methods

The data were not normally distributed; therefore, non-parametric analysis was used. Pair-wise comparisons were conducted using the Wilcoxon signed rank test. The Mann–Whitney U test has been used to compare outcomes between groups. Statistical analysis was performed using the Statistical Package for the Social Sciences program version 16.0 for Windows (SPSS v16.0, SPSS Inc.; Chicago, USA). Frequencies and percentages were used for the categorical measures. The result was considered significant if the p value <0.05.

RESULTS

Blood serum samples of patients with colorectal cancer with and without liver metastases as well as with steatosis or alcoholic cirrhosis and those of the healthy volunteers were analysed, and the GSH and GST levels were measured. Significant differences according to the patients' gender and age were observed in the groups, but neither age nor gender influenced changes in the GST and GSH levels.

The results indicating GSH and GST levels in patients with colorectal cancer (without and with liver metastases) compared to the results of healthy volunteers are summarized in Figure 1. The GSH level was statistically significantly lower in the serum of patients with colorectal cancer (without and with liver metastases) than the level in healthy volunteers (accordingly, 31.79 µg/mL vs 52.72 µg/mL, p<0.001 and 37.83 µg/mL vs 52.72 µg/mL, p<0.001). There were no statistically significant differences between the mentioned groups in terms of the GST level (accordingly, 1.25 ng/mL vs 0.53 ng/mL, p=0.747 and 0.83 ng/mL vs 0.53 ng/mL, p=0.76). Neither the GSH nor GST level statistically significantly differed among colorectal cancer patients with or without liver metastases.

However, statistically significant differences in GSH and GST levels were observed in most of the groups compared to the healthy volunteers (GSH: 52.72 µg/mL, GST: 0.53 ng/mL): with hepatic steatosis (GSH: 17.04 µg/mL, p<0.001; GST: 5.89 ng/mL, p<0.001), alcoholic cirrhosis (GSH: 62.04 µg/mL, p<0.003; GST: 0.94 ng/mL, p<0.001), liver metastases of colorectal cancer (GSH: 37.84 µg/mL, p<0.001; GST: 1.25 ng/mL, p<0.747). The highest GSH level was observed in patients with alcoholic cirrhosis, whereas the lowest was observed in patients with steatosis. The opposite results were observed for the GST level: the highest level was observed in patients with steatosis, whereas the lowest level was observed in patients with alcoholic cirrhosis (Figure 2, 3).

The GSH and GST levels were statistically significant in patients with alcoholic cirrhosis compared to the GSH and GST levels in healthy volunteers, whereas in patients with hepatic steatosis,

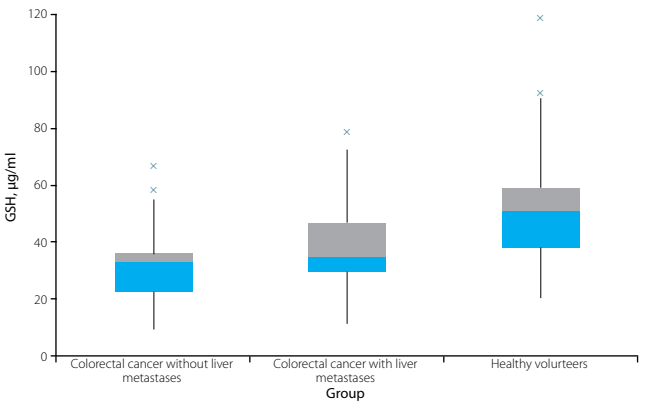

Figure 1. Reduced glutathione (GSH) level in patients with colorectal cancer with and without liver metastases

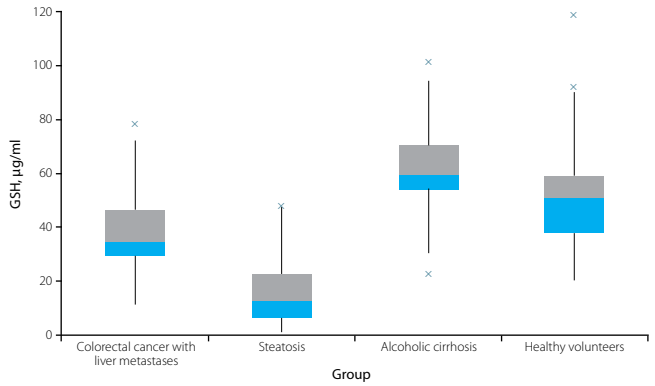

Figure 2. Reduced glutathione (GSH) level in patients with different hepatic pathologies

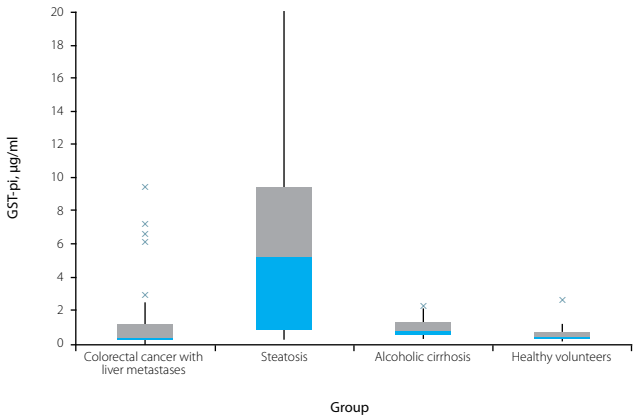

Figure 3. Glutathione-S-transferase (GST) level in patients with different hepatic pathologies

only the GST level was increased. In both cases (steatosis and colorectal cancer liver metastases), the GSH level showed a statistically significant decrease compared to the GSH level in the healthy volunteers group (Figure 2, 3).

Statistically significant differences in GSH and GST levels were observed according to the liver diseases. The GSH level in patients with alcoholic cirrhosis was higher than the GSH level in patients with steatosis as well as with liver metastases of colorectal cancer ($p<0.001$), whereas the GST level in patients with steatosis was higher than the GST level in patients with cirrhosis and in patients with colorectal cancer liver metastases ($p<0.001$).

DISCUSSION

The liver is an important site for metabolic and oxidative homeostasis, and it is involved in antioxidant activity by synthesizing major antioxidants, such as GSH (2).

Glutathione and GSH-dependent enzymes play a crucial role in the detoxification of peroxides, hydroperoxides and other free radicals. A decreased GSH level and increased GST activity demonstrate the activation of adaptive mechanisms counteracting an oxidative stress because GST uses GSH as a substrate to detoxify substances, thus inducing oxidative stress. An increased level of oxidative stress and a glutathione redox imbalance are associated with liver diseases (7,18).

It is generally known that colorectal cancer is associated with oxidative stress and an imbalance in the oxidative/antioxidative state, which result in disease progression. The GSH level and GST-dependent enzymes have been investigated in blood erythrocytes, tumour tissue, blood serum and plasma (17-22). However, the data reported in the literature on GSH and GST-dependent enzymes in colorectal cancer is controversial (23,24).

Saygili et al. (19) evaluated the differences in erythrocyte GSH levels and GSH-Px and GR and GST activities in colorectal cancer patients compared to healthy individuals. They revealed a significantly decreased erythrocyte GSH level and GSH-Px activity and increased GST and GR activities, which could be associated with antioxidant enzymatic defence mechanisms induced by oxidative stress. A decreased GST level and increased GR and SOD activity in colorectal cancer tissue were reported by Skrzydlewska et al. (20). It is worth noting that the studies by Saygili et al. (19) showed a decrease in GSH-Px activity, whereas studies by Skrzydlewska et al. (20) showed the opposite result.

Scibior et al. (17) investigated the GSH level and GSH-dependent enzymes activities in the blood serum of patients with gastrointestinal tract tumours. The GSH level was statistically significantly decreased in gastric cancer patients and increased in patients with colorectal cancer liver metastases compared to healthy blood donors, whereas the GST activity increased in patients with different gastrointestinal tract tumours before treatment compared to the GST activity of the control group.

The obtained results in our study did not confirm Scibior et al. (17) report concerning the GSH level in patients with colorectal cancer (with or without metastases). We observed a statistically significant decreased GSH level in colorectal cancer patients without metastases as well as those with metastases with respect to healthy volunteers, whereas the GST activity had a tendency to become elevated, but the difference was not statistically significant. It seems that different results could be influenced by the age, gender, size of the investigated groups and other factors (e.g. in terms of the blood sample collection after colon cancer surgery). It is important to note that the literature data of the GSH level in patients with cancer are controversial (23,24). In our study as well as in reports of others authors, a decreased GSH level and increased GST level suggest activation of the adaptive mechanisms diminishing an oxidative stress.

Because proteins, lipoproteins and other essential substances are synthesized in the liver, harmful substances are detoxicated, metabolic processes and oxidative-reductive reactions take place and oxidative stress accompanies any live pathology, be-

coming one of the most important factors disturbing liver function and structure. Therefore, there is a large amount of activity to evaluate the level of oxidative stress in different liver pathologies. Many reports indicate a decreased GSH level in patients with chronic liver diseases compared to controls (6,14), whereas the data concerning GSH-dependent enzymes activity depends on the type of enzyme. For example, in the blood serum of patients with non-alcoholic fatty liver disease and alcoholic liver disease GSH-Px, GR and catalase activity were decreased; however, SOD and GST activity were increased compared with a control group (25). We observed a decreased GSH level in the case of steatosis and an increased GSH level in the case of cirrhosis compared to the control group. In patients with colorectal cancer liver metastases, the GSH level was higher than in patients with steatosis and lower in comparison to patients with alcoholic cirrhosis. The GST activity was the highest in the patients with steatosis.

The highest GST capacity to diminish oxidative stress was determined in the case of steatosis because the GST activity was higher and the GSH level was lower than the other liver pathologies, whereas, in colorectal cancer with liver metastases patients, GST activity was lower than in patients with liver steatosis. It seems that in the case of colorectal cancer patients, oxidative stress is the most expressed and the GST capacity to detoxify substances inducing oxidative stress decreases, but it is important to note that the GST activity still remains higher than in the control group. In alcoholic cirrhosis patients, the GST activity was the lowest in comparison with the other liver pathologies. The data illustrate insufficient glutathione defence against oxidative stress.

The data indicate that the level of functional defence in the glutathione redox state against oxidative stress is different and depends on liver pathology. The expressed insufficient defence, especially in the case of alcoholic liver cirrhosis, gives evidence about the necessity to normalize disordered oxidative-reductive balance in various etiologies of liver diseases. The obtained data could be useful to correct a decreased antioxidant defence against oxidative stress.

CONCLUSION

Alterations in GSH and GST levels in patients with different liver pathologies reflect the presence of a functional defence in the glutathione redox state against oxidative stress. Decreased GSH and increased GST levels in the case of liver steatosis and liver metastases suggest activation of the adaptive mechanisms of the antioxidative system counteracting an oxidative stress, but opposite alterations found in patients with alcoholic cirrhosis showed an insufficient defence against oxidative stress.

The different GSH and GST levels in patients with colorectal cancer liver metastases, liver steatosis and alcoholic cirrhosis indicate the difference in antioxidative system damage and its compensatory possibilities and could serve as potential biomarkers for its correction.

Ethics Committee Approval: Ethics commitee approval was received for this study from the Vilnius Regional Biomedical Research Ethics Committee (2011-06-07, No 158200-06-347-88).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.B., J.D.; Design - E.B., J.D., B.K.; Supervision - J.D., E.A.; Materials - E.B., R.B., L.B.; Data Collection and/or Processing - E.B., R.B., L.B., A.K.; Analysis and/or Interpretation - B.K., A.K., L.Z.; Literature Review - E.B., L.Z.; Writer - E.B., L.Z.; Critical Review - J.D., E.A., L.B., R.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Muriel P. Role of free radicals in liver diseases. Hepatol Int 2009; 3: 526-36. [CrossRef]
2. Zhu R, Wang Y, Zhang L, Guo Q. Oxidative stress and liver disease. Hepatol Res 2012; 42: 741-9. [CrossRef]
3. Perše M. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int 2013; 2013: 725710.
4. Takaki A, Yamamoto K. Control of oxidative stress in hepatocellular carcinoma: Helpful or harmful? World J Hepatol 2015; 7: 968-79. [CrossRef]
5. Halliwell B. Free radicals and antioxidants: A personal view. Nutr Rev 1994; 52: 253-65. [CrossRef]
6. Czuczejko J, Zachara BA, Staubach-Topczewska E, Halota W, Kedziora J. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim Pol 2003; 50: 1147-54.
7. Raza H. Dual localization of glutathione S-transferase in the cytosol and mitochondria: implications in oxidative stress, toxicity and disease. FEBS J 2011; 278: 4243-51. [CrossRef]
8. Traverso N, Ricciarelli R, Nitti M, et al. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev 2013; 2013: 972913. [CrossRef]
9. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003; 22: 7369-75. [CrossRef]
10. Inokuma T, Haraguchi M, Fujita F, Tajima Y, Kanematsu T. Oxidative stress and tumour progression in colorectal cancer. Hepatogastroenterology 2009; 56: 343-7.
11. Gopčević KR, Rovčanin BR, Tatić SB, Krivokapić ZV, Gajić MM, Dragutinović VV. Activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in different stages of colorectal carcinoma. Dig Dis Sci 2013; 58: 2646-52. [CrossRef]
12. Chrobot M, Szaflarska-Szczepanik A, Drewa G. Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit 2000; 6: 713-8.
13. Broid E, Klinowski W, Koukoulis G, Hadzic N, Portmann B, Baker A, et al. Superoxide dismutase activity in children with chronic liver diseases. J Hepatol 2000; 32: 188-92. [CrossRef]
14. Loguercio C, De Girolamo V, Federico A, et al. Relationship of blood trace elements to liver damage, nutritional status, and oxidative stress in chronic nonalcoholic liver disease. Biol Trace Elem Res 2001; 81: 245-54. [CrossRef]
15. Singh M, Aggarwal HK, Aggarwal SK. Significance of The Glutathione-S Trasferase activity and total thiols status in chronic alcoholics. J Clin Diagn Res 2012; 6: 31-3.

16. Federico A, Tuccillo C, Crafa E, Loguercio C. The significance of alpha-glutathione S trasferase deteremination in patients with chronic liver diseases. Minerva Gastroenterol Dirtol 199; 45: 181-5.
17. Scibior D, Skrzycki M, Podsiad M, Czeczot H. Glutathione level and glutathione-dependent enzyme activities in blood serum of patients with gastrointestinal tract tumours. Clin Biochem 2008; 41: 852-8. **[CrossRef]**
18. Singh S, Khan AR, Gupta AK. Role of glutathione in cancer pathophysiology and therapeutic interventions. J Exp Ther Oncol 2012; 9: 303-16.
19. Saygili EI, Akcay T, Konukoglu D, Papilla C. Glutathione and glutathione-related enzymes in colorectal cancer patients. J Toxicol Environ Health A 2003; 66: 411-5. **[CrossRef]**
20. Skrzydlewska E, Stankiewicz A, Sulkowska M, Sulkowski S, Kasacka I. Antioxidant status and lipid peroxidation in colorectal cancer. J Toxicol Environ Health A 2001; 64: 213-22. **[CrossRef]**
21. Chang D, Wang F, Zhao YS, Pan HZ. Evaluation of oxidative stress in colorectal cancer patients. Biomed Environ Sci 2008; 21: 286-9. **[CrossRef]**
22. Maffei F, Angeloni C, Malaguti M, et al. Plasma antioxidant enzymes and clastogenic factors as possible biomarkers of colorectal cancer risk. Mutat Res 2011; 714: 88-92. **[CrossRef]**
23. Lin CC, Yin MC. B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. Eur J Nutr 2007; 46: 293-9. **[CrossRef]**
24. Boisio ML, Merlo F, Martines H, Esposito M. Blood levels of reduced and oxidized glutathione in malignant and non-malignant human colorectal lesions. Eur J Cancer 1990; 26: 89-91. **[CrossRef]**
25. Das KS, Balakrishnan V, Mukherjee S, Vasudevan DM. Evaluation of blood oxidative stress-related parameters in alcoholic liver disease and non-alcoholic fatty liver disease. Scand J Clin Lab Invest 2008; 68: 323-34.**[CrossRef]**