E.K. Rumyantsev, E.V. Tomtosova,V.M. Nikolaev

DOI 10.25789/YMJ.2024.86.07

UDC 577.1

GLUTATHIONE AND LIPID PEROXIDATION LEVELS IN THE BLOOD OF LUNG CANCER PATIENTS

Lung cancer is one of the most commonly diagnosed malignant tumors worldwide and is characterized by high mortality. Lipid peroxidation plays a very important role in the development and progression of lung cancer. In this regard, the purpose of this study was to evaluate the status of lipid peroxidation and the glutathione system in patients with lung cancer depending on the histological form and stage of the disease.

In this work, 100 people with lung cancer with various histological forms and stages of the disease were examined. The intensity of free radical oxidation of lipids was assessed by the accumulation of malondialdehyde. The indicators of the glutathione system were assessed by the concentration of the reduced form of glutathione and the activity of enzymes: glutathione peroxidase, glutathione reductase, glutathione transferase.

Our data indicate that as the clinical stage of lung cancer develops, the level of lipid peroxidation increases, against the background of inhibition of the glutathione system (decreased glutathione reductase activity). Higher levels of malondialdehyde in patients with adenocarcinoma suggest that tumor development may be more closely related to oxidative stress.

Keywords: lipid peroxidation, antioxidant protection, lung cancer, adenocarcinoma, squamous cell carcinoma, large cell carcinoma, small cell lung cancer.

Introduction. Lung cancer ranks first in the structure of cancer incidence worldwide [27]. This disease has one of the lowest rates of 5-year survival among oncopathologies [27]. Lung cancer is a multifactorial disease, in the development of which both exogenous (smoking, asbestos, radon, arsenic, nickel, cadmium, chromium, polycyclic aromatic hydrocarbons, chloromethyl ether, smoke from wood fuel, climatic factors, etc.) and endogenous (chronic diseases, hereditary predisposition, age hormonal shifts) factors play a significant role [21,26]. It should be noted that smoking is one of the most significant risk factors for the development of lung cancer [5]. According to Bade BC, Dela Cruz CS, 2020, if we examine lung cancer in people who have never smoked, then this disease would rank seventh in the world among oncopathologies [6].

According to many domestic and foreign authors, the initiation of free radical reactions plays an important role in the development of lung cancer [2,3,4]. Many of these exogenous and endogenous factors stimulate the development of oxidative stress in lung tissue. It should be noted that unlike other organs, due to anatomical and physiological characteristics, the lung is directly affected by a powerful oxidizer – oxygen.

Lung tissues have a fairly powerful protective system (antioxidant system) that suppresses the development of oxidative stress, the main link of which is the glutathione system. Glutathione is an intracellular tripeptide, which, in addition to antioxidant protection, performs a large number of important functions: participates in the detoxification of xenobiotics [1], promotes repair of damaged DNA [7]; provides active amino acid transport [23]; participates in the modulation of the immune response [20]; regulates the redox status of the cell [19], etc. Glutathione is a key coenzyme of enzymes (glutathione peroxidase, glutathione reductase, glutathione transferase) included in the glutathione system. The functioning of enzymes: glutathione peroxidase (an enzyme involved in the utilization of lipoperoxides and hydrogen peroxide) and glutathione transferase (an enzyme involved in the detoxification of electrophilic xenobiotics) depends on the immediate concentration of the reduced form of glutathione. The main pool of the reduced form of glutathione is supported by glutathione reductase. Information on the role of the glutathione system and lipid peroxidation, depending on the stage of the disease and the development of various histological forms of lung cancer, is scarce.

In this regard, the purpose of this study was to assess the state of the glutathione system and lipid peroxidation in the blood of lung cancer patients, depending on the histological type of tumor.

Material and methods. This work was carried out in 2024 on the premises of the Department of Epidemiology of Chronic Noncommunicable Diseases of the Yakut Science Centre of Complex Medical Problems in cooperation with the Yakut Republican Oncology Centre. In the study, 100 people with lung cancer were examined. The general characteristics of the patients are shown in Table 1.

The protocol of the study was approved by the local Committee on Biomedical Ethics at the Yakut Science Centre of Complex Medical Problems No. 52 dated 03/24/2021, decision 1.

The control group was selected based on age, gender and ethnicity. It included 60 volunteers, selected based on age. The main criterion for selection to the control group was the absence of any oncological diseases.

The study material was venous blood, which was taken on an empty stomach from the ulnar vein. The intensity of free radical lipid oxidation was determined by spectrophotometric methods based on the accumulation of TBK-active products in blood serum (TBK-AP) [11]. The following indicators of the glutathione system were determined in the hemolysate of red blood cells: glutathione peroxidase activity [17], glutathione reductase activity [10], glutathione transferase activity [14], reduced glutathione level [29]. The hemolysate of red blood cells was prepared

RUMYANTSEV Egor Konstantinovich – junior researcher, Arctic Medical Center, Yakut Science Centre for Complex Medical Problems, Yakutsk, e-mail: tzeentch1993@mail.ru, https://orcid.org/0000-0001-9843-3098; **TOMTOSOVA Eugenia Viktorovna** – junior researcher, Epidemiology of Chronic Non-Infectious Diseases Department, Yakut Science Centre of Complex Medical Problems, Yakutsk, e-mail: ytomtosova@mail.ru, https://orcid.org/0000-0002-9037-9266; **NIKOLAEV Vyacheslav Mikhailovich** – PhD in Biology, senior researcher, Epidemiology of Chronic Non-Infectious Diseases Department, Yakut Science Centre of Complex Medical Problems, Yakutsk, e-mail: Nikolaev1126@mail.ru, https://orcid.org/0000-0003-4490-8910

by diluting the purified erythrocyte mass with distilled water in a ratio of 1* 20.

Statistical processing of the obtained data was carried out using the SPSS 23 statistical software package for Windows. Standard methods of variational statistics were used: calculation of means, standard errors, 95% confidence interval. The distribution of the sample for "normality" was evaluated using the Kolmogorov-Smirnov single-sample criterion. The reliability of the differences between the means was assessed with a normal "distribution" using the Student's t criterion for independent samples, and with a different from the "normal" distribution using the Mann-Whitney criterion. The

Table 1

Brief description of the examined patients

Histological forms of lung cancer		Small cell lung cancer, n	Adenocarcinoma, n	Squamous cell lung cancer, n
All (n)		18	26	50
Sex	M	10	22	42
	F	8	4	8
Age		67.000±1.527	64.600±1.080	66.666±2.677
Stage	First	0	2	2
	Second	0	4	6
	Third	4	10	24
	Fourth	14	10	18

Table 2

The level of indicators of the glutathione and malondialdehyde system in patients with lung cancer and those without oncopathology

Groups	Control group (n=60)	Больные с онкопатологией легких			
		Stage 1 (n=4)	Stage 2 (n=12)	Stage 3 (n=40)	Stage 4 (n=44)
GPx U/ml	10.169±0.496	12.065±1.735	9.628±0.1529	11.605±1.103	11.385±1.001
GR U/ml	11.821±0.883	4.548±0.576	**2.935±0.744***	**3.024±0.342*.****	**4.179±0.570*.***.****
GST U/ml	4.050±0.066	1.488±0.504	**1.705±0.265***	**2.058±0.197***	**1.931±0.225***
GSH mcmol/L	2.285±0.072	2.965±0.206	2.704±0.199	2.205±0.365	2.148±0.404
MDA mcmol/l	1.109±0.067	1.999±0.123	**2.127±0.136***	**2.748±0.249*.****	**2.846±0.239*.*****

Note: * p<0.05 compared with the control group; ** p<0.05 compared with patients of stages 2 and 3; *** p<0.05 compared with patients of stages 2 and 4; **** p<0.05 compared with patients of stages 3 and 4.

Table 3

Indicators of the glutathione system and the level of malondialdehyde, depending on the histological form of lung cancer 1-2 stages of the disease

Histological form	GPx U/ml	GR U/ml	GST U/ml	GSH mcmol/L	MDA mcmol/l
Adenocarcinoma (n=6)	9.955±2.034	2.628±0.214	1.309±0.446	2.4233±0.303	1.958±0.198
Squamous cell lung cancer (n=8)	11.528±1.701	3.577±1.020	1.835±0.278	2.207±0.539	1.944±0.116
Small cell lung cancer (n=0)	-	-	-	-	-

Table 4

Indicators of the glutathione system and the level of malondialdehyde, depending on the histological form of lung cancer, stages 3-4 of the disease

Histological form	Control group (n=60)	Adenocarcinoma (n=20)	Squamous cell lung cancer (n=42)	Small cell lung cancer (n=18)
GPx U/ml	10.169±0.496	11.335±1.474	12.068±0.969	11.418±1.988
GR U/ml	11.821±0.883	5.218±1.096 *	4.164±0.555 *	4.324±0.554 *
GST U/ml	4.050±0.066	1.808±0.202 *	1.889±0.205 *	2.441±0.234*.**
GSH mcmol/L	2.285±0.072	2.730±0.332	2.900±0.208 *	2.885±0.283 *
MDA mcmol/l	1.109±0.067	2.913±0.051 *	2.593±0.316 *	2.295±0.205*.***

Note: * p<0.05 compared with the control group; ** p<0.05 compared with adenocarcinoma and squamous cell carcinoma; *** p<0.05 compared with adenocarcinoma.

data in the Tables are presented in the form M±m, where M is the mean, m is the error of the mean. The probability of validity of the null hypothesis was assumed at $p<0.05$.

Results and discussion. According to the data obtained, the concentrations of malondialdehyde and reduced glutathione in the blood of lung cancer patients were 1.9 times higher, p=0.010 (2.187±0.091mmol/l) and 1.2 times (2.122±1.332mmol/l), compared with people without cancer. The activity of glutathione peroxidase in the blood of patients was 1.1 times higher (11.290±0.647U/ml), and the activity of glutathione reductase and glutathione transferase were significantly lower by 2.8, p=0.000 (4.131±0.354U/ml) and 2.1, p=0.001 (1.937±0.131U/ml) times, respectively.

The data obtained by us indicate an intensification of free radical processes in the body of patients with lung cancer, as indicated by an increase in the level of the final product of lipid peroxidation – malondialdehyde and, considering the inhibition of the glutathione system, a decrease in the activity of glutathione reductase, which is consistent with literary sources [18].

The values of the biochemical parameters taken into account by us in patients, depending on the severity and in relatively healthy individuals who do not have cancer, are presented in Table 2.

The data obtained indicates that in the blood of patients with lung cancer, even in patients where the cancer is in its initial stage, the indicators of glutathione transferase and glutathione reductase activity, as well as the concentration of malondialdehyde, significantly changed compared with those without cancer. Although the indicators we take into account are not specific to this pathology, they can probably be used as additional indicators of prognostic value for early diagnosis of lung cancer. However, additional research is needed to confirm these results.

Histologically, lung cancer is divided into two groups: small cell carcinoma and non-small cell carcinoma. Non-small cell lung cancer can be divided into three histological forms: adenocarcinoma, squamous cell carcinoma and large cell carcinoma [30]. The level of biochemical parameters, taking into account the histological form and severity of the disease, is presented in Tables 3 and 4.

Due to the fact that lung cancer is very difficult to detect in its early stages, there isn't enough stage 1-2 patients to judge the statistical significance of differences in groups with various histological forms of this pathology.

In the analysis of patients with cancer stages 3-4, significant differences in the activity of glutathione reductase, glutathione transferase and malondialdehyde concentrations were revealed between lung cancer patients with different histological types and the control group (Table 4). The average value of malondialdehyde in patients with small cell lung cancer was 1.3 times lower than in patients with adenocarcinoma. In patients with small cell carcinoma, the activity of glutathione transferase was 1.3 times higher than in the group of patients with adenocarcinoma and squamous cell lung cancer. There were no statistically significant differences in other biochemical parameters between different histological forms of lung cancer.

Studies by previous authors indicated that the concentration of malondialdehyde increases in the blood of patients with lung cancer [18,28]. Some authors claim that an increase in lipid peroxidation is associated with clinical tumor progression [13,25]. The data obtained by us are consistent with the fact that as the stage of lung cancer increases, there is an increase in lipoperoxide processes in patients. However, in the study by Xiang M, Feng J, Geng L, 2019, the authors found no significant differences in the levels of total oxidant status and oxidative stress index. [31]

There are conflicting data on antioxidant protection in patients with oncopathology, some claim activation of the antioxidant system [9,15], other authors report a decrease in antioxidant protection [22,24]. Our study showed that there is no significant change in the level of the reduced form of glutathione in the blood of lung cancer patients, however, there is an inhibition of the glutathione system due to a decrease in the activity of the enzyme that restores glutathione from its oxidized form – glutathione reductase. We also noted a significant decrease in the activity of glutathione transferase. Depending on the stage of lung cancer, we noted a significant increase in the level of reduced glutathione in its initial stage by 1.3 times.

Conclusion. In conclusion, the available data indicate that as the clinical stage of lung cancer develops, during inhibition of the activity of the enzyme glutathione reductase, the level of lipid peroxidation increases. The higher level of malondialdehyde in patients with adenocarcinoma suggests that oxidative stress plays a fairly important role in the development of this tumor.

References

1. Kalinina E.V., Chernov N.N., Novichkova M.D. Rol' glutationa, glutatiotransferazy i glutaredoksina v regulyacii redoks-zavisimyh processov [The role of glutathione, glutathiotransferase and glutaredoxin in the regulation of redox-dependent processes]. Uspekhi biologicheskoj himii [Successes of biological Chemistry. 2014; 54: 299-384. EDN TUTVVJ (In Russ.).]
2. Belonogov R.N., Titova N.M., Dykhno Yu.A. [et al.]. Okislitel'naya modifikaciya belkov i lipidov plazmy krovi bol'nyh rakom legkogo [Oxidative modification of plasma proteins and lipids in patients with lung cancer]. Sibirskij onkologicheskij zhurnal [Siberian Journal of Oncology. 2009; 4 (In Russ.).]
3. Pavlov V.N., Rakhmatullina I.R., Farkhutdinov R.R. [et al.]. Svobodnoradikal'noe okislenie i kancerogenez: diskussionnye voprosy [Free radical oxidation and carcinogenesis: controversial issues]. Kreativnaya hirurgiya i onkologiya [Creative surgery and oncology. 2017; 2 (In Russ.).]
4. Abid SA "ANTI-OXIDANTS AND LUNG CANCER". Liaquat medical research journal. 2023; Jan. 4 (4) doi:10.38106/LMRJ.2022.4.4-01.
5. Andrew W.C ,Stella T, Ahmad B. (2021). Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutation Research / Reviews in Mutation Research. 2021. doi:10.1016/j.mrrev.2021.108365
6. Bade BC, Dela Cruz CS. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med. 2020;41(1):1-24. doi:10.1016/j.ccm.2019.10.001
7. Bansal A, Simon MC. Glutathione metabolism in cancer progression and treatment resistance. J Cell Biol. 2018 Jul 2;217(7):2291-2298. doi: 10.1083/jcb.201804161. Epub 2018 Jun 18. PMID: 29915025; PMCID: PMC6028537.
8. Chiang, F.-F.; Huang, S.-C.; Yu, P.-T.; Chao, T.-H.; Huang, Y.-C. Oxidative Stress Induced by Chemotherapy: Evaluation of Glutathione and Its Related Antioxidant Enzyme Dynamics in Patients with Colorectal Cancer. Nutrients. 2023; 15: 5104. https://doi.org/10.3390/nu15245104
9. Dastmalchi N, Baradaran B, Latifi-Navid S, Safaralizadeh R, Khojasteh SMB, Amini M, Roshani E, Lotfinejad P. Antioxidants with two faces toward cancer. Life Sci. 2020 Oct 1;258:118186. doi: 10.1016/j.lfs.2020.118186. Epub 2020 Aug 6. PMID: 32768586.
10. Delia A-L, Lidia M-B, Segundo Á, Nuria E-C, and et.al. The Effectiveness of Glutathione Redox Status as a Possible Tumor Marker in Colorectal Cancer . International Journal of Molecular Sciences. 2021. doi:10.3390/ijms22126183
11. D'souza D, Subhas BG, Shetty SR, Balan P. Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study. Contemp Clin Dent. 2012 Oct;3(4):448-51. doi: 10.4103/0976-237X.107438. PMID: 23633807; PMCID: PMC3636848.
12. Esme H, Cemek M, Sezer M, Saglam H, Demir A, Melek H, Unlu M. High levels of oxidative stress in patients with advanced lung cancer. Respirology. 2018 Jan;13(1):112-6. doi: 10.1111/j.1440-1843.2007.01212.x. PMID: 18197920.
13. Gurer-Orhan H, Ince E, Konyar D, Saso L, Suzen S. The Role of Oxidative Stress Modulators in Breast Cancer. Curr Med Chem. 2018;25(33):4084-4101. doi: 10.2174/0929867324666170711114336. PMID: 28699501.
14. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in

mercapturic acid formation. J Biol Chem. 1974 Nov 25;249(22):7130-9. PMID: 4436300.

15. Harris IS, DeNicola GM. The Complex Interplay between Antioxidants and ROS in Cancer. Trends Cell Biol. 2020 Jun;30(6):440-451. doi: 10.1016/j.tcb.2020.03.002. Epub 2020 Apr 14. PMID: 32303435.

16. Hiva D, Nasrin Z, Seyed A, Nahid N, Roghayeh A. "Association between Oxidative Stress Parameters and Hematological Indices in Breast Cancer Patients", International Journal of Breast Cancer, vol. 2022, Article ID 1459410, 8 pages, 2022. https://doi.org/10.1155/2022/1459410

17. Jablonska E, Gromadzinska J, Peplonska B, Fendler W, Reszka E, Krol MB, Wieczorek E, Bukowska A, Gresner P, Galicki M, Zambrano Quispe O, Morawiec Z, Wasowicz W. Lipid peroxidation and glutathione peroxidase activity relationship in breast cancer depends on functional polymorphism of GPX1. BMC Cancer. 2015 Oct 7;15:657. doi: 10.1186/s12885-015-1680-4. PMID: 26446998; PMCID: PMC4597452.

18. Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU. Oxidative stress and its role in cancer. J Cancer Res Ther. 2021;17(1):22-28. doi:10.4103/jcrt.JCRT_862_16

19. Kalinina EV, Gavriliuk LA. Glutathione Synthesis in Cancer Cells. Biochemistry (Mosc). 2020 Aug;85(8):895-907. doi: 10.1134/S0006297920080052. PMID: 33045950.

20. Kennedy L, Sandhu JK, Harper ME, Cuperlovic-Culf M. Role of Glutathione in Cancer: From Mechanisms to Therapies. Biomolecules. 2020 Oct 9;10(10):1429. doi: 10.3390/biom10101429. PMID: 33050144; PMCID: PMC7600400.

21. Malhotra, Jyoti; Malvezzi, Matteo; Negri, Eva; La Vecchia, Carlo; Boffetta, Paolo (2016). Risk factors for lung cancer worldwide. European Respiratory Journal. ERJ-00359-2016–. doi:10.1183/13993003.00359-2016

22. Moloney JN, Cotter TG. ROS signalling in the biology of cancer. Semin Cell Dev Biol. 2018 Aug;80:50-64. doi: 10.1016/j.semcdb.2017.05.023. Epub 2017 Jun 3. PMID: 28587975.

23. Ou Y, Huang J, Yang L. The prognostic significance of pretreatment serum γ-glutamyltranspeptidase in primary liver cancer: a meta-analysis and systematic review. Biosci Rep. 2018 Nov 28;38(6):BSR20181058. doi: 10.1042/BSR20181058. PMID: 30389711; PMCID: PMC6259011.

24. Poljsak B, Milisav I. The Role of Antioxidants in Cancer, Friends or Foes? Curr Pharm Des. 2018;24(44):5234-5244. doi: 10.2174/1381612825666190123112647. PMID: 30674247.

25. Sajadimajd S, Khazaei M. Oxidative Stress and Cancer: The Role of Nrf2. Curr Cancer Drug Targets. 2018;18(6):538-557. doi: 10.2174/1568009617666171002144228. PMID: 28969555.

26. Schabath MB, Cote ML. Cancer Progress and Priorities: Lung Cancer. Cancer Epidemiol Biomarkers Prev. 2019 Oct;28(10):1563-1579. doi: 10.1158/1055-9965.EPI-19-0221. PMID: 31575553; PMCID: PMC6777859.

27. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.

28. Tang X, Ding H, Liang M, Chen X, Yan Y, Wan N, Chen Q, Zhang J, Cao J. Curcumin induces ferroptosis in non-small-cell lung cancer via activating autophagy. Thorac Cancer. 2021 Apr;12(8):1219-1230. doi: 10.1111/1759-7714.13904. Epub 2021 Mar 3. Erratum in: Thorac Cancer. 2024 Apr 5;: PMID: 33656766; PMCID: PMC8046146.

29. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem. 1969 Mar;27(3):502-22. doi: 10.1016/0003-2697(69)90064-5. PMID: 4388022.

30. Wood, Steven L.; Pernemalm, Maria; Crosbie, Philip A.; Whetton, Anthony D. (2015). Molecular histology of lung cancer: From targets to treatments. Cancer Treatment Reviews, 41(4), 361–375. doi:10.1016/j.ctrv.2015.02.008

31. Xiang M, Feng J, Geng L, et al. Sera total oxidant/antioxidant status in lung cancer patients. Medicine (Baltimore). 2019;98(37):e17179. doi:10.1097/MD.0000000000017179

32. Zhang R, Kang KA, Piao MJ, Kim KC, Zheng J, Yao CW, Cha JW, Maeng YH, Chang WY, Moon P, Moon P, et al: Epigenetic alterations are involved in the overexpression of glutathione S-transferase π-1 in human colorectal cancers. Int J Oncol 45: 1275-1283, 2014