NON-PROFIT JOINT-STOCK COMPANY

"KARAGANDA MEDICAL UNIVERSITY"

ABSTRACT

of the dissertation submitted for the degree of Doctor of Philosophy (PhD) in the specialty: 6D110100 – Medicine

Indicators of Purine metabolism and Extracellular nucleic acids in the risk assessing of Chronic Respiratory Diseases development among Coal Miners with underground work txperience up to 10 years

Author: Shaukhat Diana Mukhanbetovna

Scientific Consultants: Doctor of Medical Sciences, Professor Ibrayeva Lyazat Kataevna; Candidate of Biological Sciences, Associate Professor Tankibayeva Neyla Ulanovna;

Foreign Scientific Consultant: Doctor of Medical Sciences, Professor Bukhtiyarov Igor Valentinovich (Russian Federation)

Karaganda, 2025

Relevance of the topic:

According to the Concept for the Development of Healthcare of the Republic of Kazakhstan until 2026 [1], one of the main principles of public health protection is ensuring equal access to safe, effective, and high-quality medical care.

Issues of protecting and strengthening the health of the working population are a critical problem for healthcare. The development of occupational diseases has a social character due to significant economic damage to the country and the growth of disability among individuals working in hazardous conditions [2]. In conditions that do not meet sanitary and hygienic requirements at mining enterprises and quarry development sites, every fourth employee worked in 2023 [3].

Dust generated during coal mining, when inhaled, settles in the lungs, interacts with cellular mechanisms involved in the metabolism of reactive oxygen species (ROS), damages major macromolecules (DNA, protein, and lipids), thereby stimulating a cascade of side effects, ranging from mild respiratory symptoms (cough, sputum, wheezing) to life-threatening diseases (chronic bronchitis, bronchial asthma, pneumoconiosis, pulmonary emphysema, chronic obstructive pulmonary disease (COPD)) [4].

Currently, the search for metabolic components with significant prognostic potential for the early diagnosis of chronic respiratory diseases, including dust-related lung diseases, is relevant. Purines are a group of molecules used by all cells of the body for many important biochemical processes. The role of purine metabolism is known to have a pronounced effect on cell membrane permeability, blood coagulation, prostaglandin secretion, participation in redox reactions, etc. [5, 6]. Recently, the role of extracellular purines in chronic lung diseases has been actively studied; both pro-inflammatory and protective effects of extracellular adenosine have been found. In this regard, research into the role of purine metabolites in the risk of developing chronic respiratory diseases in coal miners, depending on the duration of exposure to highly toxic anthracitic coal dust, is of interest [7-9].

The formation of occupationally induced pulmonary pathology is accompanied by additional induction of cytogenetic damage, which necessitates studying the role of extracellular DNA, RNA, and their precursors [9].

To date, the study of metabolic changes occurring in the bodies of coal miners remains relevant from the perspective of implementing preventive

measures to avert the risk of developing chronic lung diseases, which will contribute to preserving the health of the working-age population.

Aim of the study: To identify changes in indicators of purine metabolism and the concentration of extracellular nucleic acids in coal miners with underground work experience up to 10 years to assess the risk of developing chronic respiratory diseases.

To achieve this aim, the following objectives were formulated:

- **Objective 1:** To provide a clinical characterization of the coal miners with underground work experience included in the study and to assess their morbidity frequency based on electronic health passport data.
- **Objective 2:** To assess the frequency of development of chronic respiratory diseases in coal miners in dynamics 1–3 years after the start of the study.
- **Objective 3:** To study the indicators of purine metabolism and extracellular nucleic acids in the blood plasma of coal miners with underground work experience up to 10 years.
- **Objective 4:** To assess the risk of developing chronic respiratory diseases in coal miners based on multifactorial dependencies of purine metabolism and extracellular nucleic acid indicators on underground work experience.

Main points submitted for defense:

1. During the 3-year observation period from the start of the study, the development of chronic respiratory diseases was detected in coal miners: in group 1 – in 6.4%, in group 2 – in 8.4%, in group 3 – in 16.5%, in group 4 – in 8.4%.
2. Levels of metabolites of the initial stage of purine breakdown (guanine, hypoxanthine, adenine, xanthine) in the blood plasma of coal miners compared to the control group were significantly lower with underground experience up to 3 years, and became significantly higher with underground experience over 5 years.
3. Levels of extracellular RNA in the blood plasma of coal miners of all groups were significantly higher compared to the control group.
4. The main factors increasing the risk of developing chronic respiratory diseases in coal miners are an increase in the level of extracellular RNA, as well as underground work experience from 7 to 10 years.

Scientific novelty:

1. A decrease in purine metabolism metabolites (guanine, hypoxanthine, adenine, and xanthine) was first detected in coal miners with underground experience up to 3 years, with a subsequent increase with experience over 5 years (Certificate of registration of rights to a copyright object No. 39455 dated October 6, 2023, "Nature of the interaction of purine metabolism metabolites, extracellular nucleic acids, and oxidized proteins in the blood plasma of miners under the influence of the dust factor").

2. An increase in the level of extracellular RNA in the blood plasma of coal miners from the first years of underground experience, maintained subsequently for up to 10 years, was first detected (Certificate of registration of rights to a copyright object No. 39455 dated October 6, 2023, "Nature of the interaction of purine metabolism metabolites, extracellular nucleic acids, and oxidized proteins in the blood plasma of miners under the influence of the dust factor").

3. Significant indicators (increase in the level of extracellular RNA in blood plasma and underground experience from 7 to 10 years) for the early detection of the risk of developing chronic respiratory diseases in coal miners were first identified. (Certificate of registration of rights to a copyright object No. 53379, dated January 13, 2025, "Assessment of the risk of developing chronic respiratory diseases in coal miners with underground experience up to 10 years").

Practical significance of the work:

The determination of extracellular RNA in blood plasma is a diagnostic method for the early detection of the risk of developing chronic lung diseases in coal miners, which is recommended to be performed starting from 5 years of work in underground conditions. This diagnostic method has been implemented in the clinical diagnostic department of NJSC "NCLHOD" (National Center for Labor Hygiene and Occupational Diseases) (Implementation Act dated February 5, 2025).

Author's personal contribution:

The author directly participated in the analysis and synthesis of literature data, organization of material collection, and conducting all stages of the research. The author independently carried out the collection and processing of material, analysis, synthesis of research results and their description, and wrote and formatted all chapters of the dissertation work. The materials of the dissertation

work were processed and analyzed personally by the author to the extent of 95%.

Implementation into practice:

Based on the dissertation materials, 2 certificates of registration of rights to a copyright object were obtained: No. 39455 dated October 6, 2023, «Participation of purines, extracellular nucleic acids, and oxidized protein modifications in the pathogenesis of the effect of the dust factor on the organism», No. 53379 dated January 13, 2025, «Assessment of the risk of developing chronic respiratory diseases in coal miners with underground experience up to 10 years», and there are acts of implementation of the research results into the practical and scientific activities of the clinical diagnostic department of the Institute of Public Health and Occupational Health of NJSC KMU and the departments of Internal Diseases, Biomedicine, Family Medicine of NJSC KMU.

Approbation of work:

The main provisions and results of the dissertation work were presented at:

- “PhD Scientific Day” at the scientific-practical conference of young scientists "From Theory to Practice" (oral presentation “Features of purine metabolism in the blood plasma of miners”, September 20, 2019, Karaganda, NJSC KMU);
- “Modern Medicine Through the Eyes of Young Researchers” II International Scientific-Practical Online Conference (oral presentation «Уровень РНК, ДНК, АТФ и КРФ в крови Карагандинских горняков» [Level of RNA, DNA, ATP and ASF in the blood of Karaganda miners], May 20, 2021, Ingush State University, Russian Federation);
- “Influence of the Erasmus Plus Program on Increasing the Potential of Higher Education in Central Asian Universities” at the International Conference on Education and Science (oral presentation “The levels of hypoxanthine and uric acid depending on the length of the underground service of miners”, June 2-3, 2021, Almaty);
- “Occupational Medicine in the XXI Century: Health Problems of the Working Population” Scientific-Practical Conference with International Participation (poster presentation «Влияние пуринов и их метаболитов на здоровье шахтеров: данные трехлетнего наблюдения за группой» [Influence of purines and their metabolites on the health of miners: data from a three-year group observation], June 9, 2022, Karaganda);

- Scientific-Practical Conference dedicated to the 65th anniversary of the Institute of Public Health and Occupational Health with international participation “Occupational Medicine and Prospects for the Development of Medical Ecology” (oral presentation «Раннее выявление симптомов профессиональных заболеваний на примере шахтеров Карагандинской области» [Early detection of symptoms of occupational diseases using the example of miners in the Karaganda region], November 2–3, 2023, Karaganda).

List of scientific works published on the topic of the dissertation: on the dissertation materials, 4 articles were published in Russian, Kazakh, and English languages, including 3 articles in journals recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education of the MES RK, 1 article in journals with a non-zero Impact Factor and included in the Scopus database: 1 publication in international journals included in Q4 of the Scopus information database (42% at the time of publication), and 1 abstract in the proceedings of a scientific-practical conference with international participation, 2 certificates of registration of rights to a copyright object were obtained. The work underwent approbation at an international conference, 4 republican conferences with international participation, and at an expanded meeting of the Department of Internal Diseases of NJSC KMU.

Materials and methods of research:

In 2019, at the beginning of the study, the coal department of the company Joint Stock Company "ArcelorMittal Temirtau" included 18 coal mining enterprises. The number of workers engaged in underground work in hazardous conditions, particularly in contact with coal-rock dust, was 13,143 people. There were 1204 patients with occupational diseases under dispensary observation (790 per 10,000 working population).

The work is based on an observational prospective cohort study of 140 practically healthy coal miners from the Kuzembayev mine of the coal department of JSC "ArcelorMittal Temirtau". The minimum sample size calculation was performed using the "Epi InfoTM" program, which amounted to 147 people, constituting 10.9% of the total number of underground miners working at the Kuzembayev mine (1294 people). The selection of coal miners (drifters, face miners, mining machine operators, underground electricians, underground miners) for the study was carried out using the stratified sampling method during their periodic medical examination at the Medical Firm Limited

Liability Partnership "Hippocrat" in Karaganda, after signing informed consent, from June to September 2019. In the first stage, the selection of subjects was carried out according to inclusion and exclusion criteria. Then, 4 strata (groups) were defined depending on professional experience, with a minimum number of 30 people per group. Strata filling was done by simple random sampling.

In the second stage, dynamic observation was carried out with outcome assessment based on electronic health passport (EHP) data in coal miners for three years after the analysis of laboratory data.

Inclusion criteria: practically healthy coal miners, male, age 18 years and older, work experience in dusty conditions up to 10 years, absence of any radiological changes in the respiratory organs and changes in external respiration function at the time of examination, conclusion from an occupational pathologist "Practically healthy", signed informed consent. All examined individuals were informed about their inclusion in the research project and acquainted with the goals and objectives of the project. If they agreed to participate, the subjects signed an informed consent form.

Exclusion criteria: age under 18 years, female sex, presence of any pathology, and smoking.

A total of five groups (strata) were formed: 4 groups of coal miners with different work experience: Group 1 – 31 people with underground work experience up to 3 years, Group 2 – 36 people with underground work experience from 3 to 5 years, Group 3 – 37 people with underground work experience from 5 to 7 years, Group 4 – 36 people with underground work experience from 7 to 10 years. The control group (Group 0) consisted of 30 practically healthy individuals who had no contact with dust during their work activities and had no acute or chronic diseases at the time of examination.

Clinical examination was conducted according to standard methodology with completion of primary documentation, which included patient passport data, anamnesis data, physical examination data, and necessary laboratory and instrumental research methods (complete blood count, chest fluorography, spirography, electrocardiography), carried out in accordance with the "Rules for conducting mandatory medical examinations" according to the Order of the Acting Minister of National Economy of the Republic of Kazakhstan dated February 24, 2015, No. 128.

Analysis of the electronic health passport of the subjects was conducted for the years 2017–2022 to assess outcomes, frequency of seeking medical help

due to illness, and number of days absent from work on sick leave, in dynamics 1–3 years after the start of the study.

Determination of purine metabolism metabolites and extracellular nucleic acids was performed in the blood plasma of all subjects. Venous blood was used as the research material. Blood sampling was performed on an empty stomach from the cubital vein of the subject in a volume of 5 ml using sterile disposable instruments into disposable vacutainers with heparin under the conditions of a treatment room at the Medical Firm Limited Liability Partnership "Hippocrat". After centrifugation at 3000 rpm for 10 minutes, plasma was separated in the biochemical laboratory of the Department of Biomedicine of NJSC Karaganda Medical University. All rules for transportation and collection of biological material were observed during the study.

The content of free purine bases (adenine and guanine) and their catabolism intermediates – hypoxanthine, xanthine, and uric acid – in blood plasma was determined by direct spectrophotometry according to the method of Oreshnikov E.V. et al. [10]. The concentration of purine bases was expressed in extinction units (ext. units), Uric Acid (UA) – in μmol/l.

Additionally, the activity of the enzyme xanthine oxidase, a key enzyme in purine oxidation at different stages of their metabolism, was assessed. Enzyme activity was determined by calculating ratio indices: xanthine/hypoxanthine, uric acid/xanthine, uric acid/hypoxanthine. A decrease in the calculated index values compared to relative norm indicators suggests low enzyme activity, while an increase suggests high activity. The xanthine/guanine ratio was also calculated, which is an indicator of hypoxia severity. A decrease in this indicator is recorded under conditions of oxygen deficiency and indicates the presence of hypoxia [11].

As an indicator of the intensity of purine metabolism (IPM), a value was calculated representing the ratio of hypoxanthine concentration to the amount of its products (xanthine and uric acid), which determines the irreversibility of purine catabolism reactions [12]: IPM = hypoxanthine / (xanthine + Uric Acid)

Levels of extracellular nucleic acids (ecDNA and ecRNA) and their precursors - acid-soluble fractions (ASF) - were determined in the blood. To assess the degree of cellular destruction under the inductive influence of oxidative stress and inflammation, the content of extracellular nucleic acids RNA, DNA, and their ASF precursors in blood plasma was recorded according to the method of Markusheva L.I. and Savina M.I. [13]. To 1 ml of blood plasma, 5 ml of 0.5 N HClO4 was added, mixed, and then centrifuged for 15 min at 5000 rpm. The supernatant fluid, containing ASF (which includes free nucleotides,

oligonucleotides, and other nucleotide compounds – precursors of nucleic acids), was decanted into a test tube. To the sediment, 10 ml of 10% HClO4 was added, mixed, incubated at 37°C, and then centrifuged for 15 minutes at 5000 rpm. The supernatant fluid, containing the RNA hydrolysate, was decanted into a test tube. To the sediment, 3 ml of 10% HClO4 was added, mixed, incubated for 7 min at 70°C, and then centrifuged for 15 minutes at 5000 rpm. The supernatant fluid with the DNA hydrolysate was decanted into a test tube. Hydrolysates of extracellular nucleic acids (ecDNA and ecRNA) and ASF were analyzed on an Apel 303UV spectrophotometer using wavelengths of 260 and 290 nm; measurement units were determined in μg/ml.

Statistical processing of the obtained data was carried out using standard software packages for applied statistical analysis(IBM SPSS Statistics 22, Statistica 13.2) [14]. Assessment of the normality of the distribution of the obtained data was studied using the Shapiro-Wilk test, which showed a non-normal distribution of indicators. Statistical processing was carried out using non-parametric statistical methods. The Kruskal-Wallis test (for 5 independent groups) was used to compare the values of purine metabolism metabolites, extracellular nucleic acids RNA, DNA, and ASF in the blood plasma of coal miners with underground experience.

To identify differences between groups, post-hoc comparisons were made between them using the two-sample Wilcoxon test (Mann-Whitney U test). The critical significance level for them was calculated using the formula: p=1-0.95^(1/n), where n is the number of comparisons made, and amounted to p=0.0127.

The number of workers in contact with coal-rock dust at Joint Stock Company "ArcelorMittal Temirtau" in 2018 was 13,143 people. The minimum sample size calculation amounted to 147 people, conducted using the "Epi InfoTM" program, which constituted 10.9% of the total number of underground miners working at the Kuzembayev mine (1294 people) [15]. The level of statistical error was 5%.

Logistic regression was used to assess the significance of purine metabolism metabolites, ecDNA, ecRNA, and ASF plasma levels, age, duration of underground experience, duration of sick leave, on outcomes in patients of all five groups. The summary outcome group included conditions such as COPD, chronic bronchitis, chronic sinusitis, pneumoconiosis, and were coded as follows: 0 - presence of any outcome, 1 - absence of any outcome. The inclusion method

was forced entry with simultaneous inclusion of independent predictors. A critical significance level of p=0.05 was used.

Ethical approval of the study:

Ethical approval for the study was granted by the Bioethics Committee of Karaganda Medical University, protocol No. 18 dated May 16, 2019. All study participants were informed about the research objectives and signed written informed consent. Participant data were entered into a database, and participant identifiers were coded.

Conclusions:

1. From 2017 to 2019, a trend towards an increase in the number of cases was observed in group 1 from 19.4% to 32.3% and in group 2 from 22.2% to 39.9%; the main cause of morbidity in all groups of coal miners was ARVI.

2.1. In dynamics one year after the start of the study (2020), the development of chronic sinusitis (5.6%), COPD (2.8%), and pneumoconiosis (2.8%) was detected in group 4, and chronic sinusitis (5.4%) and chronic bronchitis (5.4%) in group 3 coal miners.

2.2. In dynamics two years after the start of the study (2021), radiological signs of post-COVID pneumofibrosis of the lungs (11.1%) were detected in group 3, development of chronic bronchitis (2.8%) and radiological signs of post-COVID pneumofibrosis of the lungs (5.6%) in group 2, and development of chronic sinusitis (3.2%) and chronic bronchitis (3.2%) in group 1 coal miners.

2.3. In dynamics three years after the start of the study (2022), radiological signs of post-COVID pneumofibrosis of the lungs (3.2%) were detected in group 1 coal miners.

3.1. In coal miners of group 1, a significant decrease in the concentration of metabolites of the initial stage of purine breakdown in blood plasma was observed: guanine by 9% (p=0.001), hypoxanthine by 13% (p=0.009), adenine by 11% (p=0.001), and xanthine by 13% (p=0.003) with a 4-fold increase in the level of extracellular RNA (p=0.001) relative to the control group.

3.2. In coal miners of groups 3 and 4, a significant increase in the concentration of metabolites of the initial stage of purine breakdown in blood plasma was observed: guanine by 19% and 28% (p=0.001), hypoxanthine by 10% and 21% (p=0.009), adenine by 7.5% and 25.5% (p=0.001), and xanthine by 13% and 14% (p=0.003)) with an increase in RNA level by 1.48 and 1.31 times (p=0.001) respectively relative to the control group.

3.3. In experienced coal miners of group 4, the level of uric acid content was significantly higher by 17% ($p=0.019$) compared to group 2 and by 11% ($p=0.037$) compared to group 1.

4. Based on multifactorial analysis in coal miners, it was found that the risk of developing chronic lung diseases increases 8.6 times with underground experience from 7 to 10 years ($p=0.025$), and an increase in the level of extracellular RNA content in blood plasma increases the risk 4 times ($p=0.007$).

List of references used:

1. Resolution of the Government of the Republic of Kazakhstan. On approval of the Concept for the Development of Healthcare of the Republic of Kazakhstan until 2026: approved November 24, 2022, No. 945 // https://adilet.zan.kz/rus/docs. February 19, 2025.

2. Concept of Safe Labor in the Republic of Kazakhstan until 2030 // https://www.gov.kz/memleket/entities/enbek/documents/details. February 19, 2025.

3. Number of workers employed in harmful and other unfavorable working conditions in the Republic of Kazakhstan – 2023 // https://stat.gov.kz/ru/news/chislennost-rabotnikov-zanyatykh-vo-. April 10, 2024.

4. Batool A.I., Naveed N.H., Aslam M. et al. Coal Dust-Induced Systematic Hypoxia and Redox Imbalance among Coal Mine Workers // ACS Omega. – 2020. – Vol. 5, Issue 43. – P. 28204-28211.

5. Muravlyova L.E., Molotov-Luchansky V.B., Turmukhambetova A.K. et al. Puriny v plazme krovi bol'nykh s khronicheskoy obstruktivnoy bolezn'yu legkikh razlichnoy formy i stepeni tyazhesti [Purines in the blood plasma of patients with chronic obstructive pulmonary disease of various forms and severity] // Mezhdunarodnyy zhurnal eksperimental'nogo obrazovaniya [International Journal of Experimental Education]. – 2013. – No. 10-2. – P. 263-266.

6. Senyavina N.V., Khaustova S.A., Grebennik T.K. et al. Analiz purinovykh metabolitov v syvorotke materinskoy krovi dlya otsenki riska vozniknoveniya patologii beremennosti [Analysis of purine metabolites in maternal blood serum to assess the risk of pregnancy pathology] // Byulleten' eksperimental'noy biologii i meditsiny [Bulletin of Experimental Biology and Medicine]. – 2013. – Vol. 155, No. 5. – P. 635-638.

7. Shaukhat D.M., Ibrayeva L.K., Tankibayeva N.U. Rol' purinov v razvitii professional'nykh zabolevaniy [The role of purines in the development of occupational diseases] // Nauka o zhizni i zdorov'e [Science of Life and Health]. – 2019. – No. 3. – P. 8-13.

8. Zhang R., Shao F., Wu X. et al. Value of quantitative analysis of circulating cell-free DNA as a screening tool for lung cancer: a meta-analysis // Lung Cancer. – 2010. – Vol. 69, Issue 2. – P. 225-231.

9. Wu Q., Yan W., Han R. et al. Polymorphisms in Long Noncoding RNA H19 Contribute to the Protective Effects of Coal Workers' Pneumoconiosis

in a Chinese Population // Int. J. Environ. Res. Public Health. – 2016. – Vol. 13, Issue 9. – P. 903-1-903-8.

10. Oreshnikov E.V., Oreshnikova S.F. Puriny krovi i likvora u beremennykh [Purines of blood and cerebrospinal fluid in pregnant women] // Anesteziologiya i reanimatologiya [Anesthesiology and Reanimatology]. – 2015. – No. 4. – P. 29-33.

11. Zhdanov G.G., Mikhelson V.A., Drukker N.A. Diagnostika gipoksii po urovnyu purinovykh osnovaniy krovi [Diagnosis of hypoxia by the level of blood purine bases] // Anesteziologiya i reanimatologiya [Anesthesiology and Reanimatology]. – 1982. – Vol. 27, No. 1. – P. 43-44.

12. Oreshnikov E.V., Gunin A.G., Madyanov I.V. et al. Puriny krovi i likvora vo vremya beremennosti [Purines of blood and cerebrospinal fluid during pregnancy] // Problemy reproduktsii [Problems of Reproduction]. – 2008. – No. 6. – P. 74-80.

13. Markusheva L.I., Savina M.I., Reshina V.M. et al. Yadernyye belki khromatina v otsenke effektivnosti lecheniya bol'nykh psoriazom [Nuclear chromatin proteins in assessing the effectiveness of treatment for psoriasis patients] // Klinicheskaya laboratornaya diagnostika [Clinical Laboratory Diagnostics]. – 2000. – No. 7. – P. 18-20.

14. Grzhibovsky A.M., Ivanov S.V. et al. Sravneniye kolichestvennykh dannykh trekh i boleye nezavisimykh vyborok s ispol'zovaniyem programmnogo obespecheniya Statistica i SPSS: parametricheskiye i neparametricheskiye kriterii[Comparison of quantitative data from three or more independent samples using Statistica and SPSS software: parametric and non-parametric tests] // Nauka i zdravookhraneniye [Science and Healthcare]. – 2016. – No. 4. – P. 5-37.

15. Grzhibovsky A.M., Ivanov S.V. Cohort studies in healthcare // Science and Healthcare. – 2015. – No. 3. – P. 5-16.