Mutagenesis, 2015, 00, 1–5
doi:10.1093/mutage/gev080
Original Manuscript

Original Manuscript

Modifying influence of occupational inflammatory diseases on the level of chromosome aberrations in coal miners

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Received 22 July 2015; Revised 9 September 2015; Accepted 4 November 2015.

Abstract

Coal miners are exposed to a wide range of genotoxic agents that can induce genome damage. In addition, miners are characterised by a high risk of the initiation of different occupational inflammatory as well as non-inflammatory diseases. The aim of this investigation is to analyse the modifying influence of occupational pulmonary inflammatory diseases on the level of chromosome aberrations (CAs) in miners working in underground coal mines in Kemerovo Region (Russian Federation). The study group included 90 coal miners with the following pulmonary diseases: chronic dust-induced bronchitis (CDB) and coal-workers' pneumoconiosis (CWP) (mean age = 53.52 ± 2.95 years; mean work experience in coal-mining conditions = 27.70 ± 3.61 years). As a population control (control 1), we have used venous blood extracted from 124 healthy unexposed men. The mean age in this group was 50.92 ± 4.56 years. Control 2 was the venous blood extracted from 42 healthy coal miners (mean age = 51.56 ± 6.38 years; mean work experience in coal-mining conditions = 25.43 ± 8.14 years). We have discovered that coal miners are characterised by an increased general level of CAs as well as an increased frequency of several types of CAs. The significant increase in the frequency of aberration per 100 cells and aberration of chromosome type was discovered in the group of pulmonary disease patients (study group). No correlations of the level of chromosome damage with age, smoking status and work experience in coal-mining conditions were discovered.

Introduction

Coal is one of the most abundant minerals in nature and constitutes the largest fossil fuel source used for the generation of energy (1). Coal production is prominent in various countries, e.g. the Russian Federation, Brazil, South Africa, Australia and others.

Working in coal mines, despite all achievements of modern science and technology, still remains among the most dangerous occupations to the health. Coal-mining is characterised by long-term contact with various harmful occupational agents, such as coal dust, (polycyclic aromatic hydrocarbons (PAC), ionising radiation, dampness, din and heavy metals. The influence of such agents leads not only to an increase in the risk of initiation of various chronic cardiovascular diseases, nervous diseases (vibrational angioneurosis, sensorineural hearing loss and muscle-toning syndrome) and pulmonary diseases [chronic dust-induced bronchitis (CDB), coal-workers' pneumoconiosis (CWP) and lung cancer] but also to genotoxic risk (2). Genotoxic risk is caused by the exposure to PAH, heavy metals and ionising radiation. DNA damage resulting from exposure to

genotoxic agents can be registered at the chromosomal level using methods of assessing chromosomal aberrations in human peripheral blood lymphocytes (3).

There have been a few studies of the problem of increased levels of chromosomal aberrations in coal miners in the literature, but their relationship with occupational diseases is poorly understood, and the available data are contradictory (4,5).

The large number of chronic occupational diseases in coal miners makes it necessary to investigate the bases of such diseases and to search for approaches to preventive medicine.

The occupational disease incidence leading to a temporary disability among miners in the world (including the Russian Federation) is quite high. This fact leads to economic losses among industrial factories in this sector (6,7). Despite the urgency of this problem, the association of occupational diseases and chromosome aberrations (CAs) is poorly investigated. Thus, it is highly urgent to perform the cytogenetic study of coal miners with occupational inflammatory diseases.

Materials and methods

Group characteristics

Blood samples were obtained from 90 coal miners (only men) working in the coal mines of Kemerovo Region (Russian Federation) and undergoing medical examination in the Research Institute for Complex Problems of Hygiene and Occupational Diseases (Novokuznetsk, Kemerovo Region, Russian Federation), including 31 smokers and 59 non-smokers. The study group included CDB patients (64 donors) and CWP patients (26 donors). The mean age in the study group is 53.52 ± 2.95 years; the mean work experience in coal-mining conditions is 27.70 ± 3.61 years (Table 1).

Blood samples obtained from 124 healthy unexposed men (donors of the Kemerovo Centre for Blood Transfusion), 64 smokers and 60 non-smokers, were included in control 1. The mean age was 50.92 ± 4.55 years (Table 1).

Blood samples from 42 healthy coal miners, 18 smokers and 24 non-smokers, were included in control 2. The mean age in the control 2 group was 51.56 ± 6.38 years; the mean work experience in coal-mining conditions was 25.43 ± 8.14 years (Table 1).

Miners receiving medical treatment, as well as having received an X-ray examination 3 months prior to collection of the material, who had infectious diseases or cancer were not included in the study. For each person, informed consent was obtained. The research was performed in accordance with the requirements of the Ethics Committee of Kemerovo State University.

Cytogenetic investigation

Cytogenetic investigation was performed using the routine test for the assessment of CAs (8) with certain modifications. The whole blood obtained from the ulnar vein was cultured. Volumes of 0.5 ml blood, 0.1 ml phytohaemagglutinin (PanEco, Moscow, Russian Federation), 6 ml RPMI-1640 (PanEco, Moscow, Russian Federation) and 1.5 ml embryonic veal serum (PanEco, Moscow, Russian Federation) were added to a culture flask. The duration of the cultivation was 48 h. Then, colchicine (PanEco, Moscow, Russian Federation) was added to the culture at a final concentration of 0.5 μg/ml, and the flasks were placed in an incubator for 2 h. At the end of the cultivation cycle, the preparations were centrifuged for 10 min at 1000 rpm, the supernatant was removed, and the pellet was resuspended. The pellets were placed in a hypotonic solution of 0.55% KCl for 10–15 min at 37°C. The fixation of the material was performed in cooled fresh Carnoy's fixative (methanol and acetic acid in the ratio 3:1). The cell suspension was pipetted onto clean, cooled slides moistened with water. The preparations were encoded and stained with 2% Giemsa solution.

Counting of the aberrations was performed using light microscopy at 1000× magnification (oil immersion) without karyotyping. The selection of metaphases included in the analysis and the criteria for cytogenetic abnormalities conformed to the generally accepted recommendations (9,10).

Statistical analysis

Statistical analysis was performed using the program StatSoft STATISTICA 7.0. We used the Kolmogorov–Smirnov test to verify the consistency of the data with the normal distribution. The data analysis was performed using the non-parametric statistics block. Group comparisons were performed using the U-rank Mann–Whitney test. Spearman's correlation coefficient was used to calculate a correlation.

Results

The results of the investigation of CAs in coal miners with different occupational diseases and the control groups are presented in Table 2.

The analysis of the level of CAs in the study group in comparison with control 1 showed some relationships. The frequency of the main indicator (aberrations per 100 cells) was significantly higher in the study group than in control 1 (5.72 ± 2.55% vs. 1.13 ± 1.00%). In addition, an increased frequency of various types of aberrations was discovered in the study group in comparison with control 1: single and acentric fragments, chromosome and chromatid interchanges, dicentric chromosomes, ring chromosomes, and chromatid and chromosome-type aberrations.

The comparison of the study group with control 2 (healthy coal miners) was performed to detect the genotoxic influence of certain occupational inflammatory pulmonary diseases in coal miners.

Table 1. Age, work experience and smoking status in studied groups

Group	Number	Age, years		Work experience in coal-mining conditions, years		Smoking status	
		μ ± SD	Min–max	μ ± SD	Min-max	Smoking	Non-smoking
Study group (coal miners, pulmonary diseases patients)	90	53.52 ± 2.95	45–59	27.70 ± 3.61	20–34	31	59
Coal-miners (chronic dust-induced bronchitis)	64	53.00 ± 2.63	45–58	27.73 ± 3.44	20–34	30	34
Coal-miners (coal-workers' pneumokoniosis)	26	55.09 ± 2.91	45–59	28.25 ± 3.78	21–34	8	18
Control 1 (healthy unexposed men)	124	50.92 ± 4.56	44–59	0	0	64	60
Control 2 (healthy coal miners)	42	51.56 ± 6.38	39–59	25.43 ± 8.14	4–39	18	24

Pulmonary disease patients were characterised by an increased level of chromosome interchanges at $P < 0.01$ and of dicentric chromosomes and chromosome-type aberrations at $P < 0.05$ in comparison with healthy coal miners (Table 2). Control 2 was characterised by an increased frequency of aberrations per 100 cells, single and acentric fragments, chromatid and chromosome-type aberrations and chromatid interchanges in comparison with control 1 (Table 2).

We discovered no differences in the level of CAs between CDB and CWP patients (Table 2) or between workers with different smoking status in the study group (Table 3). However, control 1 showed an increase in the frequency of dicentric chromosomes in smokers ($P < 0.01$) (Table 3).

There were no correlations of the level of cytogenetic indicators with age or work experience in coal-mining conditions.

Discussion

Kemerovo Region is the largest coal-mining area in Russian Federation, and the coal-mining industry is the leading industrial sphere in this region. According to the report of the Federal Department of National Statistics, 92 985 people (11.7% of the total working men) worked in coal mines in Kemerovo Region as of February 2015. It is known that coal workers have a high relative risk of the initiation of pulmonary diseases (in work experience of 25 years)—3.2 cases per 1000 workers. The greatest contribution to the occupational diseases in coal miners is from such diseases as CDB and CWP. The risk of initiation of certain pulmonary diseases (dusty aetiology) increases after 11 years of working in coal-mining conditions (7).

In addition to the increased risk of initiation of pulmonary and nervous diseases, as well as various personal injuries, coal miners are exposed to genotoxic risk resulting from the influence of chemical and physical agents on the organism. Cytogenetic tests permitting the detection of various types of DNA damage at the cellular and chromosomal level can be used for the assessment of such genotoxic risk. Currently, many cytogenetic tests are used for the investigation of coal miners. A number of articles describe the increased level of micronuclei (MN) and other cytogenetic abnormalities discovered using the cytokinesis-block micronucleus assay on peripheral blood lymphocytes (1,2,11), the micronucleus assay on exfoliated cells (12–14) and DNA damage discovered using the DNA-comet assay (1,2) in coal-miners in comparison with people who do not work in coal mines. Donbak *et al.* (11) used the sister chromatid exchange (SCE) test and discovered an increased level of this marker in coal miners from Turkey. Thus, we can say that miners are characterised by an increased level of cytogenetic damages associated with a considerable genotoxic influence.

In addition to genotoxic risk as a result of contact with various harmful factors, coal miners experience a risk of the initiation of various inflammatory pulmonary diseases such as CDB and CWP. It has been shown that an inflammatory process is associated with some genotoxic responses (15–17). This result indicates that pulmonary pathologies can be an additional factor inducing some types of genome damage. Thus, there is particular interest in assessing the level of cytogenetic damage in coal miners with various types of occupational diseases, to evaluate the contribution of the inflammatory process to the overall level of genotoxic responses among workers of this industry.

In this article, we have used the routine method of assessment of CAs in peripheral blood lymphocytes to estimate the level of cytogenetic damage. The number of CAs in cells is a very important quantitative parameter of mutagenesis. This indicator can be used for the assessment of certain effects resulting from the influence of genotoxic agents in coal miners. The classification of CAs is presented by Buckton (18). It is possible to determine the type of mutagenic influence according to the observed type of CAs. The increased level of dicentric and ring chromosomes may testify to a radiation source of mutagenesis (19). At the same time, chromatid-type aberrations originate from the influence of chemical mutagens. Some results reinforce the evidence of a link between CAs frequency and cancer risk and provide novel information on the role of aberration subclass and cancer type (20).

In the first stage of our investigation, we studied the frequency of CAs in the study and control groups. We detected a 10-fold excess of the frequency of ring chromosomes, a 7-fold excess of chromosome interchanges, a 6-fold excess of dicentric chromosomes, a 5-fold excess of aberrations per 100 cells, single fragments and chromosome-type aberrations, and a 4-fold excess of acentic fragments in the study group

Table 2. Results of cytogenetic analysis

	Study group (pulmonary diseases patients), μ ± SD	Control 1 (healthy unexposed men), μ ± SD	Control 2 (healthy coal miners), μ ± SD	Coal miners (chronic dust-induced bronchitis), μ ± SD	Coal miners (coal-workers' pneumokoniosis), μ ± SD
Frequency of aberrations per 100 cells	5.72 ± 2.55*	1.13 ± 1.00	5.48 ± 2.16	5.72 ± 2.59	4.58 ± 2.57
Single fragments	3.72 ± 2.15*	0.81 ± 0.79	4.15 ± 1.91	3.69 ± 2.05	2.96 ± 2.26
Chromatid interchanges	0.12 ± 0.25*	0.002 ± 0.03	0.06 ± 0.20	0.13 ± 0.27	0.09 ± 0.20
Chromatid-type aberrations	3.80 ± 2.18*	0.79 ± 0.79	4.21 ± 1.88	3.79 ± 2.08	3.04 ± 2.27
Acentric fragments	1.33 ± 0.95*	0.27 ± 0.42	1.05 ± 0.79	1.40 ± 0.97	1.28 ± 0.83
Ring chromosomes	0.10 ± 0.20*	0.01 ± 0.10	0.03 ± 0.13	0.10 ± 0.20	0.04 ± 0.14
Dicentric chromosomes	0.22 ± 0.39*	0.03 ± 0.15	0.09 ± 0.22**	0.17 ± 0.33	0.42 ± 0.57
Chromosome interchanges	0.39 ± 0.60*	0.04 ± 0.16	0.21 ± 0.35**	0.39 ± 0.47	0.92 ± 0.99
Chromosome-type aberrations	1.79 ± 1.02*	0.31 ± 0.54	1.26 ± 0.88**	1.79 ± 1.03	1.73 ± 1.18

*Significant difference in comparison with control 1, P < 0.01.

**Significant difference in comparison with study group, $P < 0.05$.

Table 3. Frequency of CAs in coal miners and healthy donors with different smoking statuses

	Study group, %, μ ± SD		Control 1, %, μ ± SD		Control 2, %, μ ± SD	
	Smoking, $N = 31$	Non-smoking, $N = 59$	Smoking, $N = 64$	Non-smoking, $N = 60$	Smoking, $N = 18$	Non-smoking, $N = 24$
Frequency of aberrations per 100 cells	5.72 ± 2.66	5.36 ± 2.78	1.11 ± 1.01	1.14 ± 1.02	5.25 ± 2.06	5.43 ± 2.17
Single fragments	3.33 ± 2.12	3.74 ± 2.15	0.73 ± 0.66	0.92 ± 0.91	3.71 ± 1.93	4.15 ± 1.67
Chromatid interchanges	0.15 ± 0.26	0.10 ± 0.24	0.004 ± 0.04	0.00	0.14 ± 0.30	0.02 ± 0.10
Chromatid-type aberrations	3.43 ± 2.11	3.82 ± 2.20	0.73 ± 0.67	0.88 ± 0.92	3.86 ± 1.87	4.17 ± 1.67
Acentric fragments	1.39 ± 0.91	1.20 ± 1.06	0.32 ± 0.48	0.19 ± 0.30	1.18 ± 0.69	1.00 ± 0.83
Ring chromosomes	0.16 ± 0.23	0.05 ± 0.15	0.008 ± 0.06	0.02 ± 0.13	0.07 ± 0.18	0.02 ± 0.10
Dicentric chromosomes	0.31 ± 0.45	0.10 ± 0.28	0.07 ± 0.20*	0.00	0.11 ± 0.21	0.08 ± 0.24
Chromosome interchanges	0.59 ± 0.47	0.37 ± 0.73	0.08 ± 0.21	0.01 ± 0.06	0.21 ± 0.25	0.24 ± 0.42
Chromosome-type aberrations	2.02 ± 0.98	1.47 ± 1.11	0.38 ± 0.63	0.21 ± 0.38	1.39 ± 0.78	1.24 ± 0.96

*Significant difference in comparison with non-smoking, $P < 0.01$.

in comparison with control 1 (Table 2). Similar results were obtained in the work of researchers from the Czech Republic(21) and Turkey (the frequency of aberrations per 100 cells was very similar to our results—5.82 ± 0.87%) (11). A research group from India published results showing the frequency of chromosome and chromatid-type aberrations to exceed our values, in the both study and control groups, but the general increasing tendency of the level of CAs was consistent with our results. This discordance can be explained by the fact that the scientists from India cultivated lymphocytes for 72 h (instead of 48 h, as in our study) and analysed a smaller number of mitosis events per person (13). Researchers from Peru (4) found a significant excess of the frequency of single and acentric fragments in a group of miners, but these indicators were lower than the similarities we have obtained for our control group. These facts can be explained because the study group studied in the work from Peru was too small (8 people).

In an article by Ulker *et al.* (22), contrasting results were reported. Three groups, healthy coal miners, CWP patients and healthy unexposed subjects, were investigated in this work. The authors described no significant differences in the level of SCEs and MN between healthy coal miners and unexposed subjects. At the same time, CWP patients were characterised by an increased frequency of cytogenetic abnormalities in comparison with healthy miners and control group. These results suggests that the inflammatory process accompanying CWP has an important influence on the level of cytogenetic indicators in coal miners.

We compared the study group with control 2 to assess contribution of the inflammatory process on the level of genetic damages. A significant excess of chromosome interchanges ($P < 0.01$) as well as dicentric chromosomes and chromosome-type aberrations ($P < 0.05$) was discovered in the study group (Table 2).

Common harmful factors that can induce the development of different diseases accompanying non-specific inflammatory processes can also result in increased expression of a genotoxic response. For example, radionuclide from radon deposits on coal particles can enter into the pulmonary epithelium and generate local radiation (23).

There is some evidence regarding the absence of significant differences between smokers and non-smokers in terms of the level of CAs, but the tendency toward an increased level of CAs in smokers was consistent with our results (11). We have not discovered any significant differences in the frequency of CAs between smokers and non-smokers. This result can be explained by the fact that a high level of CAs might be concealing a genotoxic effect from smoking (24).

Kumar *et al.* (13) showed that coal miners of 47–60 years old were characterised by a higher level of genome damages than miners younger than 47 years old. A similar relationship was discovered regarding work experience (coal miners with 25–40 years of work experience are characterised by an increased level of MN in exfoliated cells compared with miners with less than 25 years of work experience). The lack of correlation between the level of cytogenetic damage and both age and work experience in our work can be explained by the fact that most of the subjects were part of the older age group and the group with extensive work experience. A number of other studies also did not observe significant correlations between cytogenetic markers and age and work experience (1,2); an article by Donbak *et al.* (11) showed a significant correlation between the frequency of CAs and SCEs with work experience, but no relationship of these data with age was not observed.

Conclusions

The results of this work showed that coal miners are significantly characterised by increases in the total number of CAs and in the

frequency of several types of aberrations. The study of the contribution of various lung diseases of inflammatory origin to overall genotoxicity showed a significant increase in chromosomal interchanges and chromosome-type aberrations in occupational inflammatory disease patients. There was no modifying effect of smoking on the level of chromosome damage, and no correlation was found between age or work experience in hazardous conditions and the level of cytogenetic damage.

Funding

State task (No. 2015/2162).

Acknowledgements

We are particularly indebted to Dr Andrey N. Glushkov, MD (Institute of Human Ecology of SB RAS, Kemerovo, Russian Federation) for providing a database of unexposed people.

Conflict of interest statement: None declared.

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