

CHROMOSOMAL ABERRATIONS IN ORE-MINERS OF SLOVAKIA



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1. Introduction

Underground workers are exposed to various clastogenic agents. One of these agents, radon, attracts attention of recent research as it causes lung cancer in the population occupationally exposed to its various concentrations especially in mine air of uranium mines (1-3) or ore mines (4,5). Reports about higher incidence of lung cancer were coming also from ore mines of Slovakia (6-8). The chromosomal aberrations (CA) are considered to be indicators of the risk of cancerogenic effects (9). The CA are precedents of multistep cancerogenic transformation of cells (10). A positive dependence of numbers of CA from lifetime underground exposure in uranium ore miners has been reported (11). Similarly, dependence of numbers of CA from lifetime exposure to indoor radon at very low levels has been observed in inhabitants of dwellings (12).

The above information has lead us to perform a pilot study in which the numbers of CA in lymphocytes of miners of ore mines (Nižná Slaná-iron ore, Hnúšťa-talc ore) located in east central Slovakia were followed and related to the lifetime underground radon exposure and to lifetime smoking.

2. Materials and methods

Seventy miners volunteering after an informed consent served as donors of venous blood. Twenty probands which never worked underground (mostly clerks) served as donors of control blood samples.

Preparations of CA : Cultures of lymphocytes from one-hour buffy coat were started using RPMI 1640 medium supplemented with phytohaemagglutinin (0.025 ml/ml) and antibiotica. After 46 hours of incubation the suspension made from the sedimented cells was dropped upon wet slides to prepare standard specimens for microscopy. 200 well stretched mitoses have been evaluated for the presence of chromosomal and chromatid: breaks, fragments, minutes, gaps, and exchange aberrations, as well, in each of the donors.

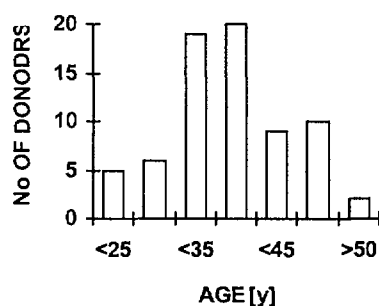
For biometrical evaluation regression analyses of the program STATGRAPHICS were used.

The exposure to radon and smoking has been estimated according to working-records and personal anamnesis.

3. Results and discussion

The age distribution of the probands is on Fig. 1. The Kolmogorov-Smirnov test showed no significant ($p>0.05$) deviation from a normal shape. The distributions of all miners including probands according to the underground exposure and according to lifetime exposure-estimates are on Table 1.

Fig 1. Age distribution of proband miners



The monthly measurements of radon concentration in the mine air were made with calibrated instruments at standard localizations. For the mine Hnúšťa the highest measured values during the years 1982 to 1994 exceeded the investigation level ($2.11E-3 \text{ mJ.m}^{-3}$) by about a factor of 3. Unfortunately, for the mine Nižná Slaná only the mean yearly concentrations of radon in air, without the range values were available. Estimates of exposure to radon were made for every mine using yearly averages of these measurements at least for last ten years before the

blood withdrawal. It was not possible to track back the exact working places and the time spent in the measured working-place for every individual miner so that strictly individual radiation body burden could not be assessed. Personal dosimetry was limited to few individuals and introduced as a trial only about 6 months before the blood withdrawal so that this could be used only tentatively for lifetime dose-estimates. The Table 1 shows that the fraction of miners in Hnúšť'a exceeding 1000 shifts underground is greater than the fraction of corresponding miners in Nižná Slaná which is reflected in higher cumulative exposure assessments in Hnúšť'a. The calculation used should be considered as the lower estimate of exposure. All what was mentioned above complicated the exposure estimates for probands. However, as the averages for ages and underground shifts of proband miners did not show statistically significant differences, and, it was felt that the error introduced by excursions of radon concentrations far exceeds the error introduced by time-averaging the exposure so that the calculation would result in a constant represented by exposure per time unit. In this alternative of calculation the numbers of underground shifts are the only variables in individual exposure estimates, so we decided to pool the groups from the two mines to attain higher counts of probands at the dividing between smokers and non-smokers, and, lower (<1500 underground shifts) or higher (>1500 shifts) groups as well as to plot the numbers of shifts underground on the x-axis of the regression graphs on Fig.2 and Fig.3.

Table 1. Distributions of miners according to underground radon shifts and exposure estimates

Plant	Parameter	Radon Shifts ^{b)}			Total ^{b)}
		< 100	< 1000	1000+	
Hnúšť'a	Workers	14	11	24	49
	Radon Shifts ^{b)}	36	339	1702	920
	Exposure ^{c)}	0.6	5.7	28.6	15.5
N.Slaná	Workers	84	175	96	355
	Radon Shifts ^{b)}	39	410	1600	644
	Exposure ^{c)}	0.6	6.9	26.9	10.8
Total ^{b)}	Workers	98	186	120	404
	Radon Shifts ^{b)}	40	405	1586	667
	Exposure ^{c)}	0.7	6.8	26.6	11.2

a) Radon Shift = Shift with concentration < 2.11 E-3 mJ.m⁻³ (<1.3 E+4 MeV.L⁻¹)

b) weighed mean

c) Exposure = mJ.h.m⁻³; WLM = 0.28 x Exposure

The values of CA numbers varied within a factor of about 6 and showed a better fit to log-normal than to normal distribution, thus, log values of counts of CA were used in regression analyses. The numeric results of the regressions are in Table 2.

Table 2. Numeric results of regression analyses

Linear regression : $y = mx + b$; $y = \ln CA$

<i>x</i>	<i>m</i>	<i>S.E.</i>	<i>p</i>	<i>b</i>	<i>S.E.</i>	<i>p</i>	<i>r</i>
lifetime shifts	-1.3x10E-5	4.4E-5	0.760	2.68	0.1	0.000	-0.03
lifetime cigarettes	1.32E-6	6.4E-7	0.044	2.56	0.06	0.000	0.24
daily cigarettes	0.0133	5.1E-3	0.011	2.53	0.06	0.000	0.30
years smoking	8.22E-3	4.0E-3	0.045	2.55	0.07	0.000	0.24
age	4.26E-4	6.3E-3	0.946	2.63	0.24	0.000	0.01

The numbers of CA found in individual donors showed a broad interindividual variation. The regression of logarithms of the numbers of CA on the numbers of lifetime underground shifts is on Fig. 2. The deviation of the slope of the regression line did not significantly differ from zero slope so that no significant influence of radon exposure on the CA counts could be ascertained.

Comparing of the means of ln of numbers of CA of miners non-smokers (2.51 ± S.D. 0.32, n=28) with those of smokers (2.75 ± S.D. 0.41, n=42) showed a statistically significant difference.

The regression of the ln of numbers of CA on the numbers of lifetime cigarettes smoked up to the time of blood withdrawal is on Fig.3. The slope of the regression line did significantly differ from zero, and the dependence on daily cigarette consumption and the dependence on time since the begin of smoking showed also such significant trend (Table 2). The dependence from age did not show a significant deviation from zero slope. This suggested that the fact of older miners smoking longer may be irrelevant to the increase of CA counts.

Table 3 and Fig.4 show the comparison of CA counts in subgroups divided according to smoking and underground exposure. A significant difference in CA counts was found between control smokers and miner-smokers exposed less than 1500 shifts underground. This suggested a contribution of the underground exposure to increase in CA counts. Two-sample tests between other combinations of these subgroups did not show a significant difference.

Fig.2. Exponential regression of CA on lifetime underground shifts

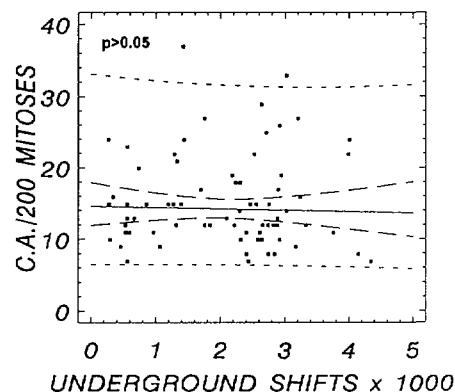


Table 3. Comparison of the ln No of CA in control and miner non-smoker and smoker groups

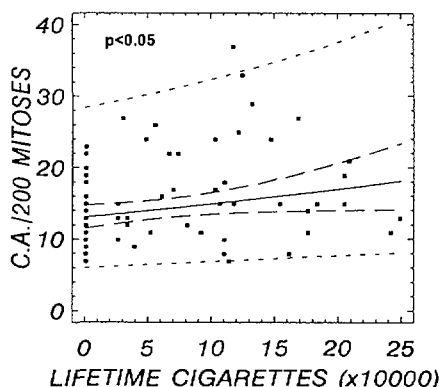
GROUP	ln No OF CA/200 MITOSES	S.D.	N	
POOLED CONTROL	2.48	0.36	20	
POOLED MINERS	2.65	0.39	70	
NON-SMOKERS				
CONTROL	2.51	0.37	12	
MINERS	2.51	0.32	28	#
MINERS < 1500 SHIFTS	2.55	0.37	10	
MINERS >1500 SHIFTS	2.47	0.29	18	
SMOKERS				
CONTROL	2.43	0.36	8	*&
MINERS	2.75	0.41	42	*#
MINERS < 1500 SHIFTS	2.78	0.37	15	&
MINERS >1500 SHIFTS	2.74	0.44	27	

*#& Differences significant, $p < 0.05$

The comparing of the numbers of CA of the exchange type (dicentrics, chromatid exchanges) pooled in subgroups using Fisher's exact test showed significant differences between control and smoker miners exposed <1500 shifts underground ($p=0.013$), as well as between non-smoker miners and smoker miners, both exposed <1500 shifts underground ($p=0.044$).

Our findings unequivocally showed a small but statistically significant clastogenic effect of the exposure to underground environment of the mines concerned. However, this pilot study does not allow a

Fig.3. Exponential regression of CA on lifetime cigarettes smoked



definite conclusion about the clastogenic potential of the professional exposure resulting from radon because other agents present in the mine environment as are dust containing clastogenic elements (Ni,Cr,As), diesel exhaust fumes, mycotoxins etc., may also have clastogenic effect. This study has shown also a small but significant influence of smoking, which in the subgroup of miners working underground less than 1500 shifts may have acted synergically with the underground exposure. Such effect was observed recently also in miners of a Namibian open-pit uranium mine (13). In order to get the results more precisely interpretable from the point of view of exposure to

radon we will in future correlate the clastogenic endpoints either to estimates of radiation effective doses from in situ measurements in relevant mines or to personal dosimeter readings (14). Nevertheless, this study indicates that it is possible to expect a lower clastogenic effect from professional underground exposure than from smoking.

4. Conclusions

- 1) Significantly higher counts of chromosomal aberrations in lymphocytes of 70 miners than in an age matched control group of 20 white-collar workers were found;
- 2) The higher counts of chromosomal aberrations could be ascribed to underground exposure of miners and to smoking;
- 3) The positive dependence of the number of chromosomal aberrations from the exposure to smoking was very loose ($r=0.24$) and it was expressed by significantly higher chromosomal aberration counts in the group of miners working less than 1500 shifts underground;
- 4) A dependence of chromosomal aberration counts from the exposure to radon could not be assessed. The low numbers of probands in subgroups caused that confounding of such dependence by smoking could not be ruled out.

5. References

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Fig.4. Comparison of averages of LN No of CA in control or miner smokers and non-smokers. Miners are divided into subgroups exposed <1500 and >1500 shifts underground

