CYTOGENETIC ANALYSIS OF COKE OVEN WORKERS IN EASTERN SLOVAKIA

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SUMMARY

Objective: Our study aimed to evaluate the extent of polycyclic aromatic hydrocarbon (PAH) exposure in coke oven workers from Eastern Slovakia by cytogenetic analysis of human peripheral lymphocytes.

Methods: A total of 81 peripheral blood samples were collected from PAH-exposed workers (mean age 45.84 ± 9.73 years) and 30 samples constituted the control group (41.93 ± 15.39 years). The samples were processed using routine cytological analysis. Conventional cytogenetic analysis of human peripheral lymphocytes has been used to evaluate the effects of PAHs.

Results: Comparison of the aberrant cells in the total exposed with the controls showed a significant difference ($p < 0.05$). A high level of significance ($p < 0.001$) was observed when comparing the gaps between the exposed group and the control group. There was a significant difference ($p < 0.01$) in aberrant cells and chromatid breaks ($p < 0.05$) in the GR1 working subgroup compared with the control group. The results of the correlation analysis did not show a significant relationship between the length of occupational exposure and the frequency of aberrant cells ($r = 0.071$, $p = 0.529$). Similarly, no association was observed between smoking among coke plant workers and the frequency of aberrant cells ($r = 0.117$, $p = 0.538$).

Conclusion: Cytogenetic analysis showed an increased frequency of chromosomal aberrations in coke oven workers in Eastern Slovakia.

Key words: cytogenetic, lymphocytes, coke oven workers, cancer, aromatic hydrocarbons

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INTRODUCTION

Industrial coking plants are a major source of emissions. They produce complex mixtures of pollutants with genotoxic, carcinogenic, and teratogenic effects, such as polycyclic aromatic hydrocarbons (PAHs) and nitrogen polycyclic aromatic hydrocarbons (nitroPAHs), which are significant environmental contaminants (1, 2). PAHs are classified as semi-volatile compounds that are released as gases or particles depending on ambient conditions (3).

Several studies have reported the negative and carcinogenic effects of PAHs. An increased skin risk has been observed with skin contact. Inhaled PAH particles can cause respiratory complications and increase the risk of lung cancer. Coke plant workers have also been reported to have a significantly higher risk of kidney and prostate cancers (4, 5).

Elevated PAH metabolite levels in the body are also associated with an increased risk of diabetes. A study by Yang et al. (6) showed that elevated urinary 4-hydroxyphenanthrene (4-OHPh) levels were significantly associated with a higher risk of diabetes in coke oven workers. This correlation was more significant in those who were overweight smokers. Alterations in humoral responses and levels of some antibodies have also been noted in this profession (7).

Higher levels of oxidative stress and tumor markers have been reported in occupationally PAH-exposed populations (8). An increased risk of stomach or gastrointestinal cancer has also been reported in the general population in association with smoked or grilled foods, such as fish and meat products, that become contaminated with PAHs during preparation (4, 5, 9). In addition, Al-Nasir et al. (10) suggested that the consumption of vegetables grown in areas where the soil is contaminated with PAHs may pose a potentially higher risk of cancer and affect human health. In addition to a direct mutagenic effect, reduced efficiency of DNA repair mechanisms has been reported in individuals exposed to PAHs, and the magnitude of this negative effect may be influenced by genetic polymorphism (11).

Cytogenetic analyses have become the gold standard in epidemiological studies for determining exposure to carcinogenic or mutagenic substances in the work environment. Chromosomal aberrations are one of the most important biomarkers for monitoring initiation of exposure and the development of carcinogenicity (12, 13). An increased incidence of chromosomal aberrations in peripheral blood lymphocytes is associated with an increased cancer risk. Chromosomal aberrations constitute a relevant and detectable marker of carcinogenesis and may predict the risk of cancer

initiation in healthy individuals (14). Owing to the increased risk of carcinogenicity (15), coke oven plants have been an area of interest for biological monitoring using cytogenetic methods for more than three decades (16, 17). Our study reports the results of cytogenetic analysis of human peripheral lymphocytes in a group of coke oven workers from Eastern Slovakia.

MATERIALS AND METHODS

Subjects and Sampling

An anamnestic questionnaire was administered to each proband before the study to determine lifestyle, dietary habits, smoking, and medical history to distinguish between the personal and investigational risk of mutagenicity. Individuals who had an infectious disease, received a vaccination, or had X-rays within 3 months before blood sample collection were excluded from the cohort due to contraindications.

The study of the effect of the presence of a chemical carcinogen on genetic material involved 81 coke plant workers. The exposed group consisted of four work groups divided according to their job descriptions. Group GR1 consisted of coke plant workers who processed of coke by-products in the plant. Group GR2 comprised workers who provided maintenance coke oven batteries. Workgroups GR3 and GR4 ensured the on-site production of coke. The control group consisted of 30 workers who worked in workplaces that were free of chemical carcinogens.

Cytogenetic Method

The 4.5 ml venous blood sample was obtained with previously heparinised syringes. Twenty-four hours after sample collection, culture was started in 20 ml culture tubes, with 0.6 ml of blood cultured in 5.83 ml of RPMI 1640 culture medium (PAN-Biotech) containing L-glutamine, $NaHCO_3$ supplemented with 1.39 ml foetal bovine serum and 0.28 ml phytohaemagglutinin (PAN-Biotech) at 37 °C. Samples were incubated for 72 h with 0.8 ml of colchicine (PAN-Biotech) added to the samples 2 h before harvest (18, 19).

Lymphocytes were collected by centrifugation and resuspended in a pre-warmed hypotonic solution (0.075M KCL) for 25 min, followed by a fixative solution consisting of methanol and acetic acid in a 3:1 ratio.

Finally, the samples were centrifuged, and the supernatant was aspirated to a residual pellet volume of approximately 0.6 ml. The sediment of the samples was mixed using an automated pipette and was dropped onto slides, which were then air-dried. The samples were stained after 24 h with 5% Giemsa-Romanowski solution.

The conventional method of cytogenetic analysis of human peripheral lymphocytes (CALPL) was used for objectification and biological monitoring of the effects of the chemicals. In the group assay, 100 mitoses were microscopically examined in each subject to determine the mean value of the percentage of chromosomal aberrations (% AB.B.). Four categories of chromosome aberrations were analyzed: chromatid, isochromatic breaks, chromatid and isochromatid exchanges. Chromatid and isochromatid gaps were not evaluated as aberrations but were included in the statistical evaluation of the groups as a separate category (19).

Statistical Analysis

The results are expressed as the mean ± standard deviation. One-factor ANOVA and unpaired t-test were used to compare the values of the exposed and control groups, and $p < 0.05$ was considered significant. Results are presented as 95% confidence intervals (CIs). Relative risk (RR) was estimated according to CA, together with 95% confidence intervals. Pearson's correlation analysis was used to determine the degree of association between the selected parameters, where $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 describes the study groups, age of the respondents, duration of occupational exposure, and the number of cells

Table 1. *General characteristics of study groups*

Subjects	n	Age Mean (SD)	Length of exposure (years) Mean (SD)	Length of smoking (years) Mean (SD)	Number of analysed cells
Exposed group					
Total	81	45.84 (9.73)	20.42 (11.18)		8,100
GR1	20	47.95 (10.11)	21.25 (12.92)		2,000
GR2	20	46.80 (9.74)	23.05 (12.03)		2,000
GR3	21	44.52 (9.61)	19.52 (8.89)		2,100
GR4	20	44.15 (9.69)	17.90 (10.76)		2,000
Smokers	30	44.53 (10.12)		17.8 (9.88)	3,000
Nonsmokers	51	46.61 (9.51)			5,100
Control group					
Total	30	41.93 (15.39)	0.00		3,000
Smokers	3	39.00 (23.07)		11.00 (8.54)	300
Nonsmokers	27	42.26 (14.91)			2,700

Table 2. *Frequency of aberrant cells*

Subjects	AB.C. (%) Mean (SD)	95% CI	p-value	RR (95% CI)
Exposed group				
Total	1.40 (1.03)	0.09–0.90	**< 0.05**	1.17 (0.88–1.54)
GR1	1.65 (1.09)	0.23–1.27	**< 0.01**	1.35 (1.01–1.81)
GR2	1.25 (0.85)	−0.11–0.81	0.135	1.20 (0.86–1.68)
GR3	1.33 (1.06)	−0.08–0.95	0.956	1.07 (0.74–1.55)
GR4	1.35 (1.14)	−0.09–0.99	0.094	1.05 (0.72–1.54)
Smokers	1.27 (0.94)	−0.68–0.27	0.342	0.91 (0.71–1.18)
Nonsmokers	1.47 (1.08)	N/A	N/A	N/A
Control group				
Total	0.90 (0.76)	N/A	N/A	N/A
Smokers	1.00 (1.00)	−0.85–1.07	0.815	N/A
Nonsmokers	0.89 (0.75)	N/A	N/A	N/A

SD – standard deviation; RR – risk ratio; CI – confidence interval; N/A – not assessed. The exposed group (total) has been compared to the control group (total). The exposed subgroups (GR1, GR2, GR3, GR4) have been compared to the control group (total). Smokers have been compared to nonsmokers within the group. Numbers in bold indicate statistically significant values.

analysed. The exposed and control groups were divided into two subgroups according to their relationship with smoking. A total of 11,100 metaphase cells were analysed.

Table 3 shows the average frequencies of the observed chromosomal aberrations in the different groups. The most frequent type of aberrations observed in the work-exposed group (total) was chromatid breaks (mean 0.89), whereas chromatid exchanges were the least represented (mean 0.01). The distribution of chromosome breaks in the exposed subgroups was as follows: in the GR1 subgroup, chromatid breaks were the most frequent aberrations (mean 1.50), and chromatid exchanges were the least frequent aberrations (mean 0.05). In subgroup GR2, isochromatid breaks were the most frequent (mean 0.70), and chromatid, isochromatid exchanges were not recorded in this subgroup. In the exposed GR3 and GR4 subgroups, chromatid breaks were the most frequent (mean 0.90 and 1.00), whereas chromatid and isochromatid exchanges were not observed in either group. In the group of exposed nonsmokers, chromatid breaks were predominant (mean 0.83), and chromatid exchanges were not observed. In the group of exposed nonsmokers, chromatid breaks were the most frequent (mean 0.92), whereas chromatid exchanges (mean 0.02) and isochromatid exchanges (mean 0.02) were the least frequent. Chromatid breaks (mean 0.50) were the most frequent aberrations in the control group; chromatid exchanges were not observed in the control group. In smokers in the control group, chromatid breaks were the most frequent aberrations (mean 0.67), as well as in the non-smoker (mean 0.48); no chromatid exchanges were observed in either group.

There was a significant difference ($p < 0.05$; RR: 1.17; 95% CI: 0.88–1.54) between the aberrant cells in the total exposed group and the control group (Table 2). When comparing the different types of breaks in the total exposed with the control, there were no significant differences ($p > 0.05$), but the difference in chromatid breaks was slightly above significance ($p = 0.057$). A high level of significance ($p < 0.001$) was observed when comparing the gaps between the exposed and control groups. There was a significant difference ($p < 0.01$; RR: 1.35; 95% CI: 1.01–1.81) in the aberrant cells and chromatid gaps between the GR1 work group and the control group ($p < 0.05$). No statistically significant differences were observed when comparing other types of aberrations. There was also a higher incidence of gaps ($p < 0.001$) in the GR1 exposed group compared than in the control group. In the other working subgroups (GR2 to GR4), there was no significant difference in the observed chromosomal aberrations compared to the control group. However, a significantly higher frequency of gaps ($p < 0.001$) was observed in the exposed GR2 and GR4 subgroups than that in the control group (Table 4). A moderate level of significance ($p < 0.01$) was observed when comparing the gap frequencies in the exposed GR3 subgroup with those in the control group. Comparisons between smokers and nonsmokers in the exposed group showed no significant differences ($p > 0.05$) between the studied categories. In addition, there was no significant difference ($p > 0.05$) in the control group when the smoking relation was monitored.

Pearson's correlation analysis showed no significant association ($r = 0.117$, $p = 0.538$) between smoking duration in the exposed group and the mean aberrant cells. There was no significant association ($r = 0.071$, $p = 0.529$) when the relationship between the length of exposure (years worked) and frequency of aberrant cells was assessed using Pearson's correlation analysis.

DISCUSSION

The frequency of aberrant cells in the study group of exposed workers (mean 1.40 ± 1.03) was higher ($p < 0.05$) than that in the control group (mean 0.90 ± 0.76). Significant differences ($p < 0.01$) in the number of aberrant cells between the work establishments and the control group were observed in the GR1 group. In this working group, chromatid breaks had the highest mean values (mean 1.50 ± 1.05). There were no significant differences in other categories of chromosomal aberrations between the exposed and the control groups. However, higher frequencies of gaps were noted in all working groups compared to the control group. The

Table 3. *Frequency of chromosomal aberrations in exposed and control groups*

Subjects	B1			B2			E1			E2		
	Mean (SD)	95% CI	p-value	Mean (SD)	95% CI	p-value	Mean (SD)	95% CI	p-value	Mean (SD)	95% CI	p-value
Exposed group												
Total	0.89 (1.05)	−0.01–0.79	0.,057	0.62 (0.77)	−0.09–0.52	0.160	0.01 (0.11)	−0.03–0.05	0.545	0.02 (0.16)	−0.08–0.06	0.805
GR1	1.05 (1.05)	0.09–1.01	**< 0.05**	0.60 (0.75)	−0.17–0.57	0.289	0.05 (0.22)	−0.03–0.13	0.224	0.10 (0.31)	−0.07–0.21	0.341
GR2	0.6 (0.60)	−0.24–0.44	0.555	0.70 (0.73)	−0.07–0.67	0.109	0.00 (0.00)		N/A	0.00 (0.00)	−0.12–0.05	0.420
GR3	0.90 (0.94)	−0.02–0.83	0.063	0.43 (0.75)	−0.34–0.40	0.877	0.00 (0.00)			0.00 (0.00)	−0.11–0.05	0.408
GR4	1.00 (1.45)	−0.09–1.09	0.095	0.75 (0.85)	−0.05–0.75	0.086	0.00 (0.00)			0.00 (0.00)	−0.12–0.05	0.420
Smokers	0.83 (0.87)	−0.57–0.39	0.717	0.43 (0.68)	−0.64–0.06	0.098	0.00 (0.00)	−0.07–0.03	0.447	0.03 (0.18)	−0.06–0.09	0.705
Nonsmokers	0.92 (1.15)			0.73 (0.80)			0.02 (1.14)			0.02 (0.14)		
Control group												
Total	0.50 (0.57)			0.40 (0.56)			0.00 (0.00)			0.03 (0.18)		
Smokers	0.67 (1.15)	−0.54–0.91	0.604	0.33 (0.58)	−0.79–0.64	0.833	0.00 (0.00)			0.00 (0.00)	−0.27–0.19,	0.745
Nonsmokers	0.48 (0.51)			0.41 (0.57)			0.00 (0.00)			0.04 (0.19)		

B1 – chromatid type breaks; B2 – isochromatid type breaks; E1 – chromatid exchange; E2 – isochromatid exchange; SD – standard deviation; CI – confidence interval. The exposed group (total) has been compared to the control group (total). The exposed subgroups (GR1, GR2, GR3, GR4) have been compared to the control group (total). Smokers have been compared to nonsmokers within the group. Numbers in bold indicate statistically significant values.

Table 4. *Frequency of gaps in exposed and control groups*

Subjects	Gaps Mean (SD)	95% CI	p-value
Exposed group			
Total	0.75 (0.81)	0.39–0.99	**< 0.001**
GR1	0.95 (0.89)	0.54–1.23	**< 0.001**
GR2	0.70 (0.66)	0.37–0.90	**< 0.001**
GR3	0.57 (0.75)	0.21–0.80	**< 0.001**
GR4	0.80 (0.95)	0.37–1.10	**< 0.001**
Smokers	0.87 (0.86)	−0.19–0.55	0.339
Nonsmokers	0.69 (0.79)	N/A	N/A
Control group			
Total	0.07 (0.25)	N/A	N/A
Smokers	0.00 (0.00)	−0.39–0.25	0.640
Nonsmokers	0.07 (0.27)	N/A	N/A

Gaps – chromatid gaps; SD – standard deviation; CI – confidence interval; N/A – not assessed. The exposed group (total) has been compared to the control group (total). The exposed subgroups (GR1, GR2, GR3, GR4) have been compared to the control group (total). Smokers have been compared to nonsmokers within the group. Numbers in bold indicate statistically significant values.

results of cytogenetic analysis of human peripheral lymphocytes indicate occupational exposure to PAHs in coke plant workers.

Several studies using cytogenetic methods have shown an increased frequency of aberrant cells in PAH-exposed groups (16, 20). Kalina et al. (1) evaluated peripheral lymphocytes from 64 coke plant workers in their biological monitoring study. Their results showed a significantly higher ($p < 0.05$) frequency of aberrant cells and breaks in the exposed group (2.30% AB.C.) compared to the control group (1.09% AB.C.). Chromatid breaks were the most common type of chromosomal aberrations reported in their study. A similar study by Ada et al. (21) aimed to determine cytogenetic damage in peripheral blood lymphocytes in Turkish coke oven workers and showed that the overall frequencies of aberrant cells without gaps were significantly higher ($p < 0.001$) in coke oven workers than in the control group. Chromosomal aberrations mainly consisted of breaks and chromatid gaps. The frequency of aberrant cells was also significantly higher in coke oven workers aged less than 40 years old ($p < 0.05$) and in those aged 41 years or older ($p < 0.01$) than in the control subgroups. Similarly, the number of aberrant cells along with gaps was significantly higher ($p < 0.001$) in the exposed group than in the control group. When comparing a subgroup of coke workers who smoked with smokers from the control group, they found a significant difference ($p < 0.001$) in the frequency of aberrant cells. In our study, it was not possible to statistically evaluate the results comparing smokers from the exposed and control groups, as there was a low representation of smokers in the control group ($n = 3$). Vimercati et al. (22), in their environmental monitoring of PAH exposure in coke oven workers, did not find a significantly higher frequency ($p > 0.05$) of aberrant cells in the exposed group compared to the control group. However, their study noted differences in the other biomarkers monitored. The exposed group had a higher frequency ($p < 0.05$) of sister chromatid exchanges than the unexposed group. P32 post-labelling analysis revealed significantly higher ($p < 0.01$) levels of PAH-DNA adducts in the

exposed group compared to the control group. The average level of the metabolic biomarker 1-hydroxypyrene was significantly higher ($p < 0.001$) in the urine of the exposed group than that of workers not working in the coke oven.

The results of our correlation analysis did not show a significant relationship between the length of occupational exposure and frequency of aberrant cells ($r = 0.071$, $p = 0.529$). Similarly, no association was observed between smoking among coke plant workers and the frequency of aberrant cells ($r = 0.117$, $p = 0.538$). Published results by Reuterwall et al. (23) linked the length of employment with a higher frequency of micronuclei. The mean age of the exposed group did not prove to be associated with the cytogenetic parameter of interest in their regression model (the association was just above significance, $p = 0.06$). Age, smoking, snuff use, and length of exposure accounted for 25% of the total variability in the number of sister chromatid exchanges. In the subgroup of smokers studied, the number of cigarettes smoked per day did not affect the mean frequency of sister chromatid exchanges. Similar to our results, Siwinska et al. (24) did not observe a correlation between the length of occupational exposure and chromosomal aberrations, nor did they observe a correlation for other cytogenetic markers. Motykiewicz et al. (20) reported a lower sensitivity of chromosome aberration analysis compared with sister chromatid exchange analysis. This fact could explain the divergence of our findings when compared with some studies (23, 25, 26). Several published studies (27, 28) using cytogenetic monitoring have demonstrated a consistent relationship between smoking and the level of DNA damage. Nevertheless, there are available publications (1, 29, 30) that have not observed a negative effect of smoking, suggesting that further research is required in this area.

CONCLUSION

We have observed differences in the average percentage of aberrant cells between the exposed groups and the control group. Significant differences were noted in the gaps category, which were more frequently noted in all exposed groups. The Pearson's correlation analysis did not show a significant relationship between the selected variables.

Our results indicate an increased risk of mutagenicity in the work environment of coke oven workers exposed to PAHs. This risk is highly associated with an increased frequency of cancer. This fact reflects the importance of prevention and the use of cytogenetic methods to objectify and monitor the risk of mutagenicity and carcinogenicity in the fields of preventive medicine and hygienic-epidemiological approaches to public health.

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Conflicts of Interest

None declared

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