Science of the Total Environment 409 (2011) 686–691

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessment of DNA damage in coal open-cast mining workers using the cytokinesis-blocked micronucleus test and the comet assay

Grethel León-Mejía [a,e], Lyda Espitia-Pérez [a,e], Luz Stella Hoyos-Giraldo [b], Juliana Da Silva [c], Andreas Hartmann [d], João Antônio Pêgas Henriques [e,*], Milton Quintana [a,*]

[a] Laboratorio de Investigación Biomédica y Biología Molecular, Universidad del Sinú, Montería, Córdoba, Colombia
[b] Department of Biology, Research Group Genetic Toxicology and Cytogenetics, Faculty of Natural Sciences and Education, Universidad del Cauca, Popayán, Cauca, Colombia
[c] Laboratorio de Genética Toxicológica (PPGGTA), Universidade Luterana do Brasil (ULBRA), Canoas-RS, Brazil
[d] Novartis Pharma AG, Basel, Switzerland
[e] Departamento de Biofísica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 31 July 2010
Received in revised form 28 October 2010
Accepted 28 October 2010

Keywords:
Coal mining
Genotoxicity
Comet assay
Micronucleus frequency
Occupational exposure

ABSTRACT

Coal mining is one of the most important causes of environmental pollution, as large quantities of coal dust particles are emitted. Colombia-South America has large natural coal reserves and "El Cerrejón" is the world's largest open-cast mine located in the northern department of Guajira. The aim of the present study was to evaluate genotoxic effects in a population exposed to coal residues from the open-cast mine "El Cerrejón". 100 exposed workers and 100 non-exposed control individuals were included in this study. The exposed group was divided according to different mining area activities: (i). Transport of extracted coal, (ii). Equipment field maintenance, (iii). Coal stripping and, (iv). Coal embarking. Blood samples were taken to investigate biomarkers of genotoxicity, specifically, primary DNA damage as damage index (DI), tail length and% of tail DNA using the Comet assay (alkaline version) and chromosome damage as micronucleus (MN) frequency in lymphocytes. Both biomarkers showed statistically significantly higher values in the exposed group compared to the non-exposed control group. No difference was observed between the exposed groups executing different mining activities. These results indicate that exposure to coal mining residues may result in an increased genotoxic exposure in coal mining workers. We did not find a correlation between age, alcohol consumption and service time with the biomarkers of genotoxicity. Our results are the first data of genotoxic effects induced by coal mining exposure in Colombia, and thus, contribute to the exploration of test batteries use for monitoring of exposed populations and may stimulate designing control, hygiene and prevention strategies for occupational health risk assessment in developing countries.

1. Introduction

Coal is one of the most abundant minerals in nature and it constitutes the largest fossil fuel source used for the generation of energy. However, its extraction and use constitute an important pollution factor which represents a major threat for human health and natural populations (Chen et al., 2005; Zakrzewski, 1991). During coal extraction large quantities of particles of coal dust are emitted contributing to environmental pollution. In addition, due to the high caloric power of this mineral, when it is exposed to ambient oxygen and sunlight a spontaneous combustion process may be initiated which liberates large amounts of Polycyclic Aromatic Hydrocarbons (PAHs) into the environment. Coal residues consists of a mixture of substances, containing carbon, hydrogen, nitrogen, oxygen and sulfur (Chen et al., 2005), mineral particles of smaller size and inorganic compounds in the ashes (UPME, 2005).

Colombia in South America has one of the world's largest natural coal reserves in Latin America and "El Cerrejón" is the world's biggest open-cast mine located in the northern department of Guajira (UPME, 2005). The main operations carried out in this mine are: stripping (extraction of coal) and crushing (mincing of coal for transporting). During the extraction processes in coal open-cast mining, particulate matters and combustion products are released into the atmosphere, where they constitute complex mixtures (Gibson, 1979). These mixtures are considered hazardous due to synergistic, additive and enhancing effects (DeMarini, 1991; Stephens and Ahern, 2001; White, 2002).

* Corresponding authors. Quintana is to be contacted at Laboratorio de Investigación Biomédica y Biología Molecular, Universidad del Sinú, Campus Elías Bechara Zainúm, Calle 38 Cra.1W Barrio Juan XXIII, Montería, Córdoba, Colombia, South America. Tel./fax: +57 4 7841961. Henriques, Departamento de Biofísica, Prédio 43422, Laboratório 210, Campus do Vale, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Bairro Agronomia-CEP 91501-970, Porto Alegre, RS, Brazil. Tel.: +55 51 33166069; fax: +55 5133167003.
E-mail addresses: pegas@cbiot.ufrgs.br (J.A.P. Henriques), quintanaso@yahoo.com (M. Quintana).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.10.049

The predominant route of coal mining residues exposure is through inhalation. Today it is known that chronic inhalation of complex mixtures, containing substances such as heavy metals, ash, iron, PAHs and sulfur, can result in lung disorders including simple pneumoconiosis, progressive massive fibrosis, bronchitis, loss of lung function, emphysema and even cancer (Beckman and Ames, 1997; Schins and Borm, 1999). Recent studies have postulated that some of these diseases may be a consequence of inhaling such material resulting in activation of macrophages, interaction with epithelial cells and other cells, finally leading to generation of oxidative stress (Kamp et al., 1992). Besides direct cellular damage, compounds released by coal mining activities such as PAHs present an important mutagenic hazard that has been associated with an increased risk for cancer development (Mastrangelo et al., 1996). Despite these findings, coal dust remains classified as non carcinogen for human (Group 3) in International Agency for Research on Cancer (IARC, 1997).

Biomonitoring studies in peripheral lymphocytes of coal workers demonstrated increased adduct formation and increased of non-cellular and cellular sources of reactive oxygen species that can induce oxidative DNA damage (Schins et al., 1995; Schoket et al., 1999).

There are only a few studies on the occupational hazard effects in coal miners. However, some studies have been conducted in animals of mining regions. For example, cytotoxic and genotoxic effects of coal dust have been evaluated *in vivo* in wild rodents in coal mining areas in Brazil (Da Silva et al., 2000a,b) and in Colombia (León et al., 2007).

The aim of the present study was to evaluate potential genotoxic effects in peripheral blood lymphocytes in an exposed population to coal residues in the open-cast mine "El Cerrejón" in Guajira-Colombia using Comet assay and Micronucleus (MN) test. The results obtained from this study present the first data for Colombia on a genotoxic hazard generated by coal mining residuals exposure. In addition, our data represent an assessment of the feasibility of using tool biomarkers for evaluating potential occupational health risks. An area of application of such methods is to investigate whether it can be applied in coal mining activities in Colombia to support efforts for applying cleaner production procedures which would have less negative impacts on the environment and the human population.

2. Materials and methods

2.1. Individuals and sampling

The exposed individuals included in this study were workers from "El Cerrejón" open-cast coal mine, in Guajira Department in the north coast of Colombia, South America. A group of 100 exposed individuals engaged in surface activities of the sub-bituminous open-cast coal mine with a minimum time of service of 5 years were selected. The non-exposed control group consisted of 100 individuals, from Guajira department, with no known exposure to genotoxic agents including coal, radiation, chemicals or cigarettes. Both study populations (exposed and non-exposed group) lived in the same region considering that the two populations should have the same genetic background and the same life habits.

The exposed group was divided according to the engaged activities in the mine: (i) *Extracted coal transport* ($n=50$) in which the workers are involved in coal transport up to the arrival in the storing centers. (ii) *Equipment field maintenance* ($n=18$) these workers drive trucks to spread water in the roads where large quantities of coal dust are generated the workers also fix the coal extraction equipment. (iii) *Coal stripping* ($n=17$) these workers are engaged in coal stripping activities and the accumulation of the material for the transport in trucks, they also extinguish fires generated by coal spontaneous combustion. (iv) *Coal embarking* ($n=15$) these workers are involved in shipping of coal in containers to be exported to other countries.

Exposed workers were matched to non-exposed controls by age (± 2 years) and similar social-economic status. Confounding and exclusion factors were collected from all participants who responded to an interviewer-administered, detailed, standard questionnaire which included data of health status, cancer history, other chronic diseases, lifestyle, nutrition, smoking habits, medication intake, and frequency of alcohol consumption (total number of drinks and the most widely alcoholic beverages consumed), occupational and time of service, protective measures, and previous exposure to medical X-rays or treatment with known carcinogens. All individuals included into the study were non-smokers. The exposed group was selected according to the following inclusion approaches: voluntary acceptance, been healthy and time of service ≥ 5 years. Exclusion criteria for exposed and non-exposed groups were age over 60 years or less than 24 years, smoking (current and ex-smoking habits), medical treatment up to 3 months or X-ray up to 1 year before sampling and therapeutic drugs intake, known to be mutagenic. All data was organized and recorded in databases. There are no major differences regarding social-economic status or dietary habits were identified.

The whole study population was informed about the aim, benefits, risks and methodology details of the study though the informed consent, which was obtained from all individuals. We previously had the approval of the University of Sinú Ethic Committee for this research study. All the information identifying the study individuals is kept at the "Laboratorio de Investigación Biomédica y Biología Molecular" of the University of Sinú in the city of Montería, Cordoba department, which is the only institution having full identifiable information about the individuals.

2.2. Blood samples collection

After informed consent was obtained from each individual, peripheral blood samples from all 200 individuals were collected by venipuncture. 20 mL of blood were drawn into heparin tubes (Becton Dickenson, vacutainer) for the Comet assay and MN test. All blood samples tubes were coded and kept in upright position at room temperature in dark during the transportation overnight to the laboratory, where the samples were processed immediately upon arrival.

2.3. Comet assay

The Comet assay in the present study was carried out according to the original methodology (alkaline version) described by Singh et al. (1988) and Singh and Pfeifer (1996) with slight modifications (Heuser et al., 2007; Da Silva et al., 2008). 30 μL of isolated lymphocytes by Histopaque 1077, were mixed with 270 μL 0.5% of low melting point (LMA-Invitrogen) at 37 °C. This mixture was placed into a slide previously coated with 1.5% of normal melting point agarose (NMA-Cambrex Bioscience Rockland) processed at 60 °C. The agarose layers were covered with a cover slip and after gel solidifying the cover slips were removed. The slides were immersed overnight in lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5, 1% with freshly added 1% Triton X-100 and 10% DMSO) at 4 °C in dark. Afterwards, the slides were placed for 30 min in alkaline buffer at 4 °C (300 mM NaOH and 1 mM EDTA, pH>13) to unwind the DNA. The alkaline electrophoresis was carried out for 30 min at 25 V and 300 mA. This standard alkaline procedure allows single-strand DNA breaks to be detected and alkali labile lesions (i.e., apurinic/apirimidinic sites) are converted to strand breaks under these conditions as well. The gels were neutralized with 0.4 M Tris (pH 7.5) with 3 washes of 5 min each. Finally, the slides were stained with 50 μL ethidium bromide (2 μL/mL) and examined at 40× magnification under a fluorescence microscope equipped with a green filter of 540 nm. Direct light exposure of the samples was avoided during the whole process. For each individual we analyzed 100 randomly

selected Comets (50 cells from each of two replicate slides). For the analysis of the images we used a system image analysis software and the considered endpoints as measurement to quantify DNA damage, were tail length and % of tail DNA. In addition, the cells were classified according to tail size into five classes ranging from undamaged (0) to maximally damaged (4), obtaining a measure of the individual damage for each animal and consequently for each analyzed group. The damage index (DI) calculation was carried out according to the visual classification system (Collins et al., 1997). The values for the damage index could range from 0 (100 cells class 0) up to 400 (100 cells class 4). For the statistical analysis we took into account the mean values for all the Comet assay parameters.

2.4. Micronucleus test

The MN test using the cytokinesis-block technique was performed in this study (Fenech and Morley, 1985; Fenech, 1993; Heuser et al., 2007; Da Silva et al., 2008). This approach allows reliable data scoring because only the MN of those cells that have completed one nuclear division are analyzed. Cultures were prepared with whole blood in duplicate and processed as described by Albertini et al., 2000. Heparinized whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 medium (Sigma R8758, USA) supplemented with 2 mM L-glutamine (Sigma A5955,USA), 10% fetal bovine serum (Gibco/Invitrogen 15000-044, Brazil), 100 μL/mL antibiotic–antimycotic (Sigma A5955, USA) and 2% phytohemaglutinin (Sigma L8754, USA) to stimulate the lymphocytes. Cultures were incubated at 37 °C in dark for 46 h, under 5% CO_2 in a humidified atmosphere. Two parallel cultures were set in tubes (Falcon 3033) for each sample. Cytocalasin B (Sigma, C6762) was added at 44 h of incubation at a final concentration of 6 μg/mL. The cells were harvested at 72 h, treated with hypotonic solution (0.075 M KCl) immediately centrifuged and fixed three times with methanol/acetic acid (3:1). The fixed cells were dropped onto humidified slides and air dried slides were stained with Giemsa for 10 min. MN registration was performed on coded slides with double blind. Two thousand binucleated cells (BN) per individual (1000 BN per culture) were registered. All coded slides were analyzed with an optical light microscope (40× magnification). All slides were scored by one reader blinded to the exposure status of the individuals. The scoring criteria followed those proposed by Fenech et al., 2003.

2.5. Statistical analysis

The normality of the variables was evaluated using the Kolmogorov–Smirnov test; $\chi 2$ and *t*-tests were used to compare the demographic characteristics of study populations. The statistical analysis of differences in MN test and DNA damage measured by Comet assay were carried out using the non-parametric Mann Whitney *U*-test and statistical differences between the five groups (non-exposed control, extracted coal transport, equipment field maintenance, coal stripping, and coal embarking) were analyzed using the non-parametric two-tailed Kruskal–Wallis test with the Dunn correction for multiple comparisons to perform a non-parametric analysis of variances. Correlations between different variables were determined by Spearman rank correlation test as appropriate. The critical level for rejection of the null hypothesis was considered to be *p* value of 5%. All analyses were performed with the PRISMA 5.0 statistical software package.

3. Results

The main demographic characteristics of the study population are shown in Table 1. The mean age of exposed group was 44.0 ± 7.5 years (range, 24–60 years), and non-exposed control group was 43.7 ± 7.8 years (range, 27–60 years).The mean time of service ± standard deviation (SD) of the exposed group was 17.7 ± 6.9 years (range, 5–30 years).

Table 1
Main demographic characteristics of the studied population: non-exposed control and exposed groups.

Demographic characteristics	Groups	
	Non-exposed control	Exposed
Number of individuals	100	100
Age (mean ± SD)	43.7 ± 7.8	44.0 ± 7.5
Time of service (Mean years ± SD)	–	17.7 ± 6.9
Alcohol consumption		
Non-alcohol consumers	55 (55%)	45 (45%)
Alcohol consumers*	45 (45%)	55 (55%)

SD = Standard deviation.
* Drink more than three beers/day or drink in excess of once a week.

Table 1 shows the percentage of alcohol consumption for non-exposed group (45%) and exposed group (55%), considered as alcohol consumer that drink more than three beers per day or drink in excess of once a week.

Table 2 summarizes the Comet assay data and Micronucleus frequency values for both study groups. The mean values of both biomarkers parameters in the exposed group demonstrated significant differences when compared to the values of the non-exposed control group ($p<0.001$), which were analyzed using Kruskal–Wallis test (Dunn correction). There was no statistically significant difference between the four different coal mining activities ($p>0.05$).

The results obtained in Micronucleus frequency show that the values in the exposed to coal mining residuals group (8.6 ± 4.8) are higher compared with the non-exposed control group (2.9 ± 4.0). The differences were significant as is shown in Table 2, evaluated through Mann Whitney *U*-test ($p<0.001$).Table 2 shows the mean values of the Comet assay parameters of the two study groups. This comparison, for each Comet assay parameter damage index, tail length and% of tail DNA, clearly demonstrates higher levels of DNA damage in the exposed group (mean tail length = 23.4 ± 6.5; mean% of tail DNA = 13.1 ± 7.9; mean DI = 60.0 ± 39.5) compared to the non-exposed control group (mean tail length = 14.3 ± 2.5; mean% of tail DNA = 2.9 ± 1.5; mean DI = 9.0 ± 6.4). These differences were all statistically significant evaluated using Mann Whitney *U*-test ($p<0.001$).

The Spearman correlation coefficients for MN frequency with age, and DNA damage (tail length, % of tail DNA, DI) for non-exposed control and exposed groups were not significant ($p>0.05$). The correlations between MN frequency with time of service, and DNA damage (tail length, % of tail DNA and DI) for the subdivided exposed groups, were not significant ($p>0.05$). We analyzed the effect of alcohol consumption on MN frequencies and DNA damage in all groups using Mann Whitney *U*-test and found no difference in any of the groups ($p>0.05$).

4. Discussion

Occupational exposure to coal residues is a public health concern in developing countries owing to the lack of regulation policies and epidemiologic surveillance programs and to the limited coal combustion residues and particulate matter emission management (Popovic et al., 2001; Baba and Kaya, 2004). During coal open-cast mining extraction processes significant amounts of these substances are released to the atmosphere where they constitute complex mixtures (Gibson, 1979). The main route of exposure to these particulate matters is through inhalation. Most of the published studies have focused on underground coal workers (Knudsen et al., 2005; Donbak et al., 2005) and only a few studies like Sram et al. (1985) have evaluated induction of chromosome aberrations by coal complex

Table 2
Comet assay and Micronucleus test parameters in non-exposed control and Exposed group divided by work areas activities (mean ± standard deviation).

Parameters	Non-exposed control group ($n=100$)	Whole exposed group ($n=100$)	Exposed group/work areas activities			
			Extracted coal transport ($n=50$)	Equipment field maintenance ($n=18$)	Coal stripping ($n=17$)	Coal embarking ($n=15$)
Comet assay (100 lymphocytes/individual)						
Tail length	14.3 ± 2.5	23.4 ± 6.5*	22.9 ± 7.1*	23.6 ± 6.0*	25.1 ± 7.1*	22.2 ± 3.7*
% of Tail DNA	2.9 ± 1.5	13.1 ± 7.9*	12.0 ± 7.9*	13.5 ± 7.7*	16.5 ± 9.4*	11.8 ± 6.1*
Damage index (index range = 0–400)	9.0 ± 6.4	60.0 ± 39.5*	55.9 ± 40.1*	62.7 ± 38.2*	74.0 ± 45.9*	53.8 ± 31.0*
Micronucleus test frequency (2000 binucleated cells/individual)	2.9 ± 4.0	8.6 ± 4.8*	7.9 ± 4.2*	8.6 ± 5.0*	8.5 ± 4.7*	11.0 ± 5.8*

* Significant difference in relation to the non-exposed control group; Kruskall Wallis–Dunn correction $p<0.001$.

mixtures in open-cast mining workers. Colombia possesses the largest coal reserves in Latin America (UPME, 2005); however, to date, genotoxic endpoints related to coal open-cast extraction has only been evaluated in rodent populations (León et al., 2007).

In the present study we investigated potential genotoxic effects of coal exposure in mining workers. Biological monitoring of exposure to chemical substances in the workplace is crucial to assess potential human health risks, as an integral strategy to improve occupational health, safety conditions and life quality in developing countries. We applied the Comet assay to compare the extent of primary DNA damage and the Micronucleus test as cytogenetic effect biomarker in peripheral blood lymphocytes from exposed and non-exposed individuals.

Our results demonstrate that the group exposed to coal mining residuals exhibited a significantly higher extent of DNA damage in peripheral lymphocytes in the Comet assay and MN test compared to the control group. Previous studies with underground coal mine workers have demonstrated higher levels of chromosomal damage, evaluated through chromosomal aberration, MN and sister chromatid exchange assays (Donbak et al., 2005; Sram et al., 1985; Santa Maria et al., 2007; Agostini et al., 1996). There are no significant differences in DNA damage average (Comet assay and MN test) of control group were detected between this study and previous studies of our group (Heuser et al., 2007; Da Silva et al., 2008).

It is known that coal mining activities release significant quantities of fugitive particles and toxic gases as sulfur dioxide into the environment (UPME, 2005). Some quality parameters of coal from the open-cast mine "El Cerrejón" are total moisture (~10%), volatiles (~30%), ash (~8%), sulfur (~1%), carbon (~70%), hydrogen (~6%), oxygen (~5%), nitrogen (~1%), as well as different metals (ETSU & Department of Trade and Industry, 2000). Chronic exposure to this coal complex mixture release into the atmosphere (Gibson, 1979) constitute one of the most important occupational health and safety risks of workers due to the potential synergistic toxic effects of this compound mixture (White, 2002; DeMarini, 1991). Considerable data has suggested that reactive oxygen species (ROS) and their products are involved in the pathogenesis of lung disorders and cancer risk (Schins et al., 1995; Cooke et al., 2003; Beckman and Ames, 1997) in exposed workers. The primary target cells of inhaled coal dust particles are macrophages and epithelial cells. Activated macrophages (phagocytosis toxicity) produce excessive amounts of ROS and cytokines. ROS may additionally be generated via cell-independent mechanisms due to intrinsic chemical properties of the coal dust (e.g. surface radicals and iron). Epithelial cells and fibroblasts which are the main producers of components of the extracellular matrix including collagens, proteoglycans and elastic fibres, are also known to produce cytokines and ROS upon stimulation. Additional phagocytotic cells (neutrophils, monocytes/macrophages) may be recruited by chemokines produced by the alveolar macrophages as well as epithelial cells, and may amplify local production of ROS and cytokines. Both ROS and cytokines may cause damage or proliferation of local epithelial and mesenchymal tissue and may as such have consequences to lung tissue morphology, cell turnover and deposition of extracellular matrix components. The formation and degradation of extracellular matrix may also be affected by ROS, as well as proteases and antiproteases produced by the various cell types present in the surrounding tissue (Schins and Borm, 1999). When there is excessive production of ROS, or when there are insufficient *in vivo* defense mechanisms, oxidative stress may occur. This stress may result in DNA damage, lipid peroxidation, protein modification, membrane disruption, and mitochondrial damage (Zhai et al., 2002), all modifications capable of affecting cytogenetic damage levels.

Other components associated to heavy metals toxicity such as Copper, Lead, Cadmium, Nickel, Vanadium and Zinc appear in high concentrations in coal complex mixture (ATSDR, 1993). Large amounts of mutagenic compounds such as PAHs are produced during the spontaneous coal combustions which is a common process in coal storing centers due to environmental factors as sunlight and climate conditions. Some PAHs related to coal mining such as chrysene and benzo(k)fluoranthene are indicators of coal combustion, and pyrene and fluoranthene are associated with such processes (Sai, 1995). PAHs can induce DNA lesions as single-strand breaks via DNA repair mechanisms (Pavanello et al., 2005; Rojas et al., 2000; Brescia et al., 1999) and electrophilic metabolites that covalently interact with the DNA (Pereira-Netto et al., 2000; Singh et al., 2007) forming adducts with purines, especially with guanine after metabolic activation by enzymatic complex P450 (Baird et al., 2005).

Due to the complex mixture in occupational coal mining environments it is difficult to relate genotoxic effects to a specific agent or compound. The Comet assay is particularly sensitive towards the detection of direct and indirect DNA strand breakage and DNA alkaline-labile sites. These types of DNA damages are usually induced by most of the genotoxic agents which induce DNA breaks at the phosphodiester skeleton or between bases and sugars resulting in abasic sites (Tice et al., 2000). The MN assay in peripheral blood lymphocytes is extensively used in biomonitoring and molecular epidemiology studies to evaluate the presence and the extent of chromosomal damage in exposed human populations to genotoxic agents, and also because an increased MN frequency has been validated as a cancer risk biomarker in humans (Fenech, 1999; Bonassi et al., 2007).

In our study no significant difference in markers of genotoxicity between the four coal mine activities (extracted coal transport, equipment field maintenance, coal stripping and coal embarking) was observed. These observations indicate that exposed workers have a comparable genotoxic response to complex mixture exposure independently to the area and activity in the mine. In addition to these findings, there was no correlation between the time of service and Comet assay parameters or MN frequency. This observation is in agreement with the results obtained in other occupational settings studies (Sailaja et al., 2006; Gomez-Arroyo et al., 2000).

In our results, there was no influence of alcohol consumption, age or time of service in the exposed individuals on Comet assay parameters tail length, % of tail DNA and damage index or the MN frequency (Celik et al., 2007; Shaham et al., 2002; Pinto et al., 2000). The results obtained with respect the alcohol consumption are in

agreement with other occupational studies in which there was no association between the alcohol consumption and the levels of DNA damage observed (Rajah and Ahuja, 1995; Martinez-Valenzuela et al., 2009; Sailaja and Satyaprasad, 2006).

In conclusion, while we did not specifically verify the underlying mechanisms of the genotoxic effects observed in our study, DNA damage in the exposed mining workers may be a consequence of oxidative damage resulting from coal residue mixtures containing traces of iron, sulfur, coal ash, heavy metals and PAHs. Our results are the first data for the genotoxic damage induced by coal mining exposure for Colombia and contribute to the assessment of the usefulness of such biomarkers. Our results may motivate towards the use of biomarkers to monitor hygiene and prevention strategies in occupational settings in developing countries.

Acknowledgments

This study was supported by COLCIENCIAS 1283-408-20487/2008 and by Vicerrectoría de Investigaciones Universidad del Sinú and Universidad del Cauca, Colombia. We are grateful with Jaime Luna, Shirly Salcedo, Ingrid Reyes, Jorge Galeano, Luisa Fernanda Escobar-Hoyos, Dra. Temenouga Guecheva, Nubia Yandar, Victoria Jaramillo, Hugo Brango and Paula Rohr for their invaluable help. We thank the help of SINTRACARBON — "El Cerrejón" study population and to the non-exposed controls for their great disposition during the sampling.

References

Agostini J, Otto P, Wajntal A. Chromosome damage in underground coal miners: detection by conventional cytogenetic techniques and by submitting lymphocytes of unexposed individuals to plasma from at-risk groups. Braz J Genet 1996;19:641–6.

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Int Program Chem Saf Mutat Res 2000;463:111–72.

ATSDR. agency for toxic substances and disease registry-ToxFAQs Toxicological profile for: Lead, Cadmium, Nickel, Vanadium and Zinc. University of Utah; 1993. Available on line. Website:http://www.atsdr.cdc.gov/toxfaq.html.

Baba A, Kaya A. Leaching characteristics of solid wastes from thermal power plants of western Turkey and comparison of toxicity methodologies. J Environ Manage 2004;73:199–207.

Baird WM, Hooven LA, Mahadevan B. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ Mol Mutagenesis 2005;45:106–14.

Beckman KB, Ames BN. Oxidative decay of DNA. J Biol Chem 1997;272:19633–6.

Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis 2007;28:625–31.

Brescia G, Celotti L, Clonfero E, Neumann HG, Forni A, Foà V, et al. The influence of cytochrome P450 1A1 and glutathione S-transferase M1 genotypes on biomarker levels in coke-oven workers. Arch Toxicol 1999;73:431–9.

Celik M, Donbak L, Unal F, Yuzbasioglu D, Aksoy H, Yilmaz S. Cytogenetic damage in workers from a coal-fired power plant. Mutat Res 2007;627:158–63.

Chen Y, Shah N, Huggins FE, Huffman GP. Transmission electron microscopy investigation of ultrafine coal fly ash particles. Environ Sci Technol 2005;39:1144–51.

Collins AR, Dobson VL, Dusinska M, Kennedy G, Stetina R. The comet assay: what can it really tell us? Mutat Res 1997;375:183–93.

Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 2003;17:1195–214.

Da Silva J, de Freitas T, Heuser V, Marinho J, Bittencourt F, Cerski C, et al. Effects of chronic exposure to coal in wild rodents (Ctenomys torquatus) evaluated by multiple methods and tissues. Mutat Res Genet Toxicol Environ Mutagen 2000a;470:39–51.

Da Silva J, de Freitas T, Heuser V, Marinho J, Erdtmann B. Genotoxicity biomonitoring in coal regions using wild rodent Ctenomys torquatus by comet assay and micronucleus test. Environ Mol Mutagen 2000b;35:270–8.

Da Silva J, Moraes CR, Heuser VD, Andrade VM, Silva FR, Kvitko K, et al. Evaluation of genetic damage in a Brazilian population occupationally exposed to pesticides and its correlation with polymorphisms in metabolizing genes. Mutagenesis 2008;23:415–22.

DeMarini D. Environmental mutagens/complex mixtures. Genet Toxicol 1991:285–302.

Donbak L, Rencuzogullari E, Yavuz A, Topaktas M. The genotoxic risk of underground coal miners from Turkey. Mutat Res 2005;588:82–7.

ETSU & Department of Trade and Industry. The fate of trace elements in PF combustion systems. Report No. COAL R193 - DTI/Pub URN 00/947http://webarchive.nationalarchives.gov.uk/tna/+/http://www.dti.gov.uk/files/file18569.pdf/; 2000.

Fenech M. The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. Mutat Res 1993;285:35–44.

Fenech M. Micronucleus frequency in human lymphocytes is related to plasma vitamin B12 and homocysteine. Mutat Res 1999;428:299–304.

Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res 1985;147:29–36.

Fenech M, Bonassi S, Turner J, Lando C, Ceppi M, Chang WP, et al. Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes: results of an international slide-scoring exercise by the HUMN project. Mutat Res Genet Toxicol Environ Mutagen 2003;534:45–64.

Gibson J. Coal — an introduction to its formation and propierties. In: Pitt GJ, Millward GR, editors. Coal and Modern Coal Processing: An Introduction. Academic Press; 1979. p. 1-25.

Gomez-Arroyo S, Diaz-Sanchez Y, Meneses-Perez MA, Villalobos-Pietrini R, De Leon-Rodriguez J. Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides. Mutat Res 2000;466:117–24.

Heuser VD, Erdtmann B, Kvitko K, Rohr P, da Silva J. Evaluation of genetic damage in Brazilian footwear-workers: biomarkers of exposure, effect, and susceptibility. Toxicology 2007;232:235–47.

IARC, International Agency for Research on Cancer, Agents Classified by the IARC Monographs, Volumes 1–100. Cas N° 000050-41-9. 1997; 68.

Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 1992;12:293–315.

Knudsen LE, Gaskell M, Martin EA, Poole J, Scheepers PT, Jensen A, et al. Genotoxic damage in mine workers exposed to diesel exhaust, and the effects of glutathione transferase genotypes. Mutat Res 2005;583:120–32.

León G, Perez LE, Linares JC, Hartmann A, Quintana M. Genotoxic effects in wild rodents (*Rattus rattus* and *Mus musculus*) in an open coal mining area. Mutat Res 2007;630:42–9.

Martinez-Valenzuela C, Gomez-Arroyo S, Villalobos-Pietrini R, Waliszewski S, Calderon-Segura ME, Felix-Gastelum R, et al. Genotoxic biomonitoring of agricultural workers exposed to pesticides in the north of Sinaloa State, Mexico. Environ Int 2009;35:1155–9.

Mastrangelo G, Fadda E, Marzia V. Polycyclic aromatic hydrocarbons and cancer in man. Environ Health Perspect 1996;104:1166–70.

Pavanello S, Pulliero A, Siwinska E, Mielzynska D, Clonfero E. Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B[a]PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. Carcinogenesis 2005;26:169–75.

Pereira-Netto AD, Moreira JC, Dias AEXO, Arbilla G, Ferreira LFV, Oliveira AS, Barek J. Avaliação da Contaminação Humana por Hidrocarbonetos Policíclicos Aromáticos (HPAS) e seus Derivados Nitrados (NHPAS): Uma Revisão Metodológica. Quim. Nova 2000;23:765–73.

Pinto D, Ceballos JM, Garcia G, Guzman P, Del Razo LM, Vera E, et al. Increased cytogenetic damage in outdoor painters. Mutat Res 2000;467:105–11.

Popovic A, Djordjevic D, Polic P. Trace and major element pollution originating from coal ash suspension and transport processes. Environ Int 2001;26:251–5.

Rajah T, Ahuja YR. In vivo genotoxic effects of smoking and occupational lead exposure in printing press workers. Toxicol Lett 1995;76:71–5.

Rojas M, Cascorbi I, Alexandrov K, Kriek E, Auburtin G, Mayer L, et al. Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 2000;21:35–41.

Sai XC. The pollution of PAH. Environ Protect 1995;10:31–3.

Sailaja KK, Satyaprasad K. Degradation of glyphosate in soil and its effect on fungal population. J Environ Sci Eng 2006;48:189–90.

Sailaja N, Chandrasekhar M, Rekhadevi PV, Mahboob M, Rahman MF, Vuyyuri SB, et al. Genotoxic evaluation of workers employed in pesticide production. Mutat Res Genet Toxicol Environ Mutagen 2006;609:74–80.

Santa Maria SR, Arana M, Ramirez O. Chromosomal aberrations in peripheral lymphocytes from male native miners working in the Peruvian Andes. Genet Mol Biol 2007;30:1135–8.

Schins RP, Borm PJ. Mechanisms and mediators in coal dust induced toxicity: a review. Ann Occup Hyg 1999;43:7-33.

Schins RP, Schilderman PA, Borm PJ. Oxidative DNA damage in peripheral blood lymphocytes of coal workers. Int Arch Occup Environ Health 1995;67:153–7.

Schoket B, Poirier M, Mayer G, Török G, Kolozsi-Ringelhann Á, Bognár G, et al. Biomonitoring of human genotoxicity induced by complex occupational exposures. Mutat Res Genet Toxicol Environ Mutagen 1999;445:193–203.

Shaham J, Gurvich R, Kaufman Z. Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. Mutat Res 2002;514:115–23.

Singh NP, Pfeifer GP. Microgel electrophoresis of DNA from individual cells: Principles and Methodology. Technologies for Detection of DNA Damage and Mutations. New York: Plenum Press; 1996. p. 3-24.

Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.

Singh R, Sram RJ, Binkova B, Kalina I, Popov TA, Georgieva T, et al. The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. Mutat Res Fundam Mol Mech Mutagen 2007;620:83–92.

Sram RJ, Hola N, Kotesovec F, Vavra R. Chromosomal abnormalities in soft coal open-cast mining workers. Mutat Res 1985;144:271–5.

Stephens C, Ahern M. Worker and Community health impacts related to mining operations internationally: a rapid review of the literature, Mining, Minerals and Sustainable

Development Project (MMSD). Institute for Environment and Development (IIED), World Business Council, for Sustainable Development (WBCSD); 2001. p. 59.
Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000;35:206–21.
UPME. Plan de expansión de referencia de generación-transmisión (Versión preliminar). In: www.upme.gov.co, editor. Vol. 2019, 2005.
White P. The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. Mutat Res Genet Toxicol Environ Mutagen 2002;515:85–98.
Zakrzewski S. Principles of environmental toxicology. Washington, DC: ACS; 1991.
Zhai R, Liu G, Ge X, Yang C, Huang C, Wu C, et al. Genetic polymorphisms of MnSOD, GSTM1, GSTT1, and OGG1 in coal workers' pneumoconiosis. J Occup Environ Med 2002;44:372–7.