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Genetic damage in coal miners evaluated by buccal micronucleus cytome assay

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ABSTRACT

During coal mining activities, large quantities of coal dust, ashes, polycyclic aromatic hydrocarbons and metals are released into the environment. This complex mixture presents one of the most important occupational hazards for health of workers. The aim of the present study was to evaluate the genetic damage together with the presence of inorganic elements, in an exposed workers population to coal mining residues of Guajira-Colombia. Thus, 100 exposed workers and 100 non-exposed control individuals were included in this study. To determine genetic damage we assessed the micronucleus (MN) frequencies and nuclear buds in buccal mucosa samples (BMCyt) assay, which were significantly higher in the exposed group than non-exposed control group. In addition, karyorrhectic and karyolytic cells were also significantly higher in the exposed group (cell death). No significant difference was observed between the exposed groups engaged in different mining activities. No correlation between age, alcohol consumption, time of service and MN assay data were found in this study. However, the content of inorganic elements in blood samples analyzed by a Particle-induced X-ray emission technique (PIXE) showed higher values of silicon (Si) and aluminum (Al) in the exposed group. In this study we discuss the possibility of DNA damage observed in the mine workers cells be a consequence of oxidative damage.

1. Introduction

It is known that coal mining activities are a major source of environmental contamination. Mining activities release large amounts of substances that can form complex mixtures containing CO_x, NO_x, SO_x, aluminum silicon crystals, quartz, metals (arsenic, boron, cadmium, chromium, lead, copper, selenium, iron, and zinc), and polycyclic aromatic hydrocarbons (PAH) into the environment (Zhou et al., 2005).

The main route of coal mining exposure to these potentially hazardous residues is by inhalation of coal dust particles from the extraction and manipulation activities. Currently, it is known that chronic inhalation of coal dust particles can result in lung disorders including simple pneumoconiosis, progressive massive fibrosis, bronchitis, lung function loss, emphysema and cancer. Studies were able to establish that some of these disorders could have their origin in genetic damage generated by the inhalation of mineral particles. In particular interaction of particles with macrophages, epithelial cells and other cells could lead to generation of reactive oxygen species (ROS) (Schins and Borm, 1999; Cooke et al., 2003).

The effects of coal exposure have been studied using bacteria (Nakajima et al., 2008), bats (Zocche et al., 2010), rodents (Da Silva et al., 2000, León et al., 2007) and human cells (Celik et al., 2007; Rohr et al., 2013a, 2013b). Some studies in workers exposed to coal mining residues assessed by chromosomal aberrations (Santa Maria et al., 2007), sister chromatid exchange, and micronuclei

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(MN) in peripheral blood lymphocytes (Donbak et al., 2005; León-Mejía et al., 2011) demonstrated that occupational exposure to coal dust can lead to a significant induction of cytogenetic damage. In a previous study, we found elevated DNA damage in coal mining workers from Guajira-Colombia, assessed by the Comet assay and MN test in lymphocytes (León-Mejía et al., 2011). Despite these findings, coal dust remains classified as "not classifiable as to its carcinogenicity to humans" (Group 3) by the International Agency for Research on Cancer (IARC, 1997).

The fact that a very high percentage of cancers have an epithelial origin suggests that micronuclei in epithelial cells are an important biomarker that can be used for epidemiological studies. Micronuclei that are detected in exfoliated buccal cells reflect genotoxic events that occurred in basal cells, and these events can be observed in exfoliated cells over an approximately three week period (Holland et al., 2008). The buccal micronucleus cytome assay (BMCyt assay) is considered a fast and simple method for *in situ* biomonitoring of human populations exposed to environmental genotoxicans (Majer et al., 2001; Bonassi et al., 2011).

The aim of the present study was to evaluate the genotoxic effects in exfoliated buccal cells and concentrations of inorganic elements in a population exposed to coal residues in the open-cast mine "El Cerrejón" in Guajira-Colombia using the buccal micronucleus cytome assay (exfoliated buccal cells; BMCyt assay) and the particle-induced X-ray emission (PIXE) in blood samples. The MN data in buccal were compared to MN data in lymphocytes from our previous study (León-Mejía et al., 2011) to assess whether buccal cells can be used as a non-invasive source to investigate biomarkers of genetic damage in exposed individuals.

2. Materials and methods

2.1. Individuals and sampling

This study was approved by the Committee on Research Ethics at University of Sinú Ethic and details of the study through the informed consent were obtained from each individual before the research began.

This study involved a total of 200 individuals, who live in the same region in order to ensure a comparable genetic background and life habits. The exposed group were 100 workers occupationally exposed to coal with a minimum time of service of 5 years in "El Cerrejón" open-cast coal mine, in the Guajira Department in the north coast of Colombia, South America. The non-exposed control group consisted of 100 individuals with no known exposure to genotoxic agents including coal, radiation, chemicals or cigarettes. Both study populations (exposed and non-exposed groups) lived in the same region; it was considered that the two populations should have presented the same genetic background and the same life habits.

The workers were involved in different activities in the mine: (i) *transport* of *extracted coal* ($n=50$), in which the workers are involved in coal transport up to arrival in the storing centers; (ii) *equipment field maintenance* ($n=18$), these workers drive trucks to spread water onto the roads where large quantities of coal dust are generated, and also maintain 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 spontaneous combustion of coal; (iv) *coal embarking* ($n=15$), these workers are involved in shipping of coal in containers to be exported to other countries. All workers were exposed to large quantity of coal dust, but was perceived that the coal stripping group was the most exposed to coal mining residues.

All individuals in the study were required to answer a questionnaire and participate in a face-to-face interview, which included determination of standard demographic data and questions concerning medical issues (exposure to X-rays, vaccinations, medication, *etc.*), life style (smoking, alcohol consumption, diet, *etc.*), cancer history, other chronic diseases and occupation (number of working hours per day, protective measures adopted). All individuals included in the study were non-smokers and have time of service ≥ 5 years. Buccal cell and blood samples were obtained from all individuals.

2.2. Buccal micronucleus cytome assay (BMCyt assay)

After informed consent was obtained from each individual, buccal mucosa samples from all 200 individuals were collected. The subjects were asked to rinse their mouth with water before sampling. The exfoliated buccal mucosa cells were collected using a cytobrush to gently scrape the mucosa of the inner lining of both cheeks. All buccal sample tubes were coded and kept in upright position at room temperature.

The cells were washed three times in 0.9 percent phosphate saline buffer, the smears were made from the pellet and fixed in methanol:acetic acid (3:1). For microscopic analysis, the slides were incubated at 37 °C overnight and then stained with Giemsa (Stich and Rosin, 1984; Acar et al., 2001). The frequency of MN was determined in 2000 cells for each person following recommendations of Thomas et al. (2009). All slides were scored by one reader blinded to the exposure status of the individuals.

MN and other nuclear abnormalities were classified according to Tolbert et al. (1992) and Thomas et al. (2009). Nuclear anomalies, such as karyorrhectic and karyolytic cells (different forms of cell death), and nuclear buds (indicative of gene amplification) were assessed in 2000 cells/individual and recorded separately.

2.3. Particle-induced X-ray emission (PIXE)

Peripheral blood samples from all 200 individuals were collected by venipuncture. Thus, 5 mL of blood were drawn into heparin tubes (Becton Dickenson, vacutainer) for the particle-induced X-ray emission (PIXE) analysis. All blood samples tubes were coded and kept at room temperature. Blood samples were analyzed for the total content of metals by the particle induced X-ray emission (PIXE) technique (He et al., 1993; Johansson et al., 1995). This technique has been successfully employed to detect trace elements in plants and animals because of its multielemental character, high sensitivity, simplicity and high sample throughput (Mireles et al., 2004).

For the analyses, the blood samples were dried at 40 °C for 72 h, then macerated using a mortar, and finally pressed into pellets which were positioned on the target of the reaction chamber. A 3 MV Tandetron accelerator provided 2.0 MeV proton beams with an average current of 5 nA at the target. The X-rays induced by the beam in the samples were detected by a Si(Li) detector with an energy resolution of about 155 eV at 5.9 keV. The spectra were analyzed with the GUPIXWIN software package (Maxwell et al., 1995; Campbell, 2000) and the final results are expressed in parts per million (ug g^{-1}). The chemical elements analyzed in the samples by the PIXE method were: sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), bromine (Br) and rubidium (Rb). The organic matrix of the blood (the organic composition of the sample) was determined by the Rutherford Backscattering Spectrometry (RBS) technique.

2.4. Statistical analysis

The normality of the variables was evaluated using the Kolmogorov–Smirnov test; χ^2 and *t*-tests were used to compare the demographic characteristics of study populations and chemical elements analyzed by PIXE. The statistical analysis of differences in MN frequency between the exposed and control group 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. Correlations between MN frequency in lymphocytes obtained in our previous study (León-Mejía et al., 2011) and MN frequencies in buccal cells of the present study in control and exposed individuals were determined by Spearman rank correlation test. The critical level for rejection of the null hypothesis was considered to be $P<0.05$. All analyses were performed with the PRISMA 5.0 statistical software package.

3. Results

The mean age and standard deviation 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 of the exposed group was 17.7 ± 6.9 years (range, 5–30 years). The percentage of alcohol consumption for non-exposed group was 45 percent and for exposed group was 55 percent, considering as alcohol consumer to drink alcohol in excess of once/week.

Table 1 summarizes the values of the MN frequencies for both study groups, exposed and control groups, with exposed group differentiated by the mining area activities. There was no statistically significant difference between the different mining area activities ($P>0.05$; Kruskal–Wallis test), however the micronuclei frequencies observed to each individual subgroup exposed to coal mining were significantly increased compared to control group values ($P<0.05$; Kruskal–Wallis test).

Table 1
Parameters of genetic damage (micronuclei), gene amplification (nuclear bud) and cell death (karyorrhexis and karyolitic cells) observed in exfoliated buccal of the control and exposed group divided by mining area activities (mean ± standard deviation).

Groups	Micronucleus test parameters			
	Micronucleus	Nuclear bud	Karyorrhexis	Karyolytic
Control (*n*=100)	1.0 ± 2.2	1.0 ± 1.1	35.8 ± 30.5	25.3 ± 17.3
Exposed (*n*=100)	8.8 ± 12.8[a]	2.7 ± 4.2[b]	64.8 ± 54.9[b]	36.1 ± 40.1[b]
Exposed per mining area				
Transport of extracted coal (*n*=50)	8.9 ± 11.7[c]	2.3 ± 2.9[c]	63.1 ± 49.0[c]	30.3 ± 38.4
Equipment field maintenance(*n*=18)	8.7 ± 14.9[c]	1.6 ± 2.3	64.4 ± 56.0[c]	36.3 ± 37.6[c]
Coal stripping (*n*=17)	11.3 ± 17.7[c]	4.1 ± 6.5[c]	85.7 ± 78.3[c]	38.2 ± 68.8[c]
Coal embarking (*n*=15)	5.7 ± 6.1[c]	3.0 ± 4.3[c]	52.0 ± 33.7[c]	29.0 ± 16.1

[a] $P < 0.001$, Mann–Whitney *U*-test.
[b] Significant difference compared to the control group at $P < 0.05$.
[c] Significant difference compared to the control group at $P < 0.05$, Kruskal Wallis test.

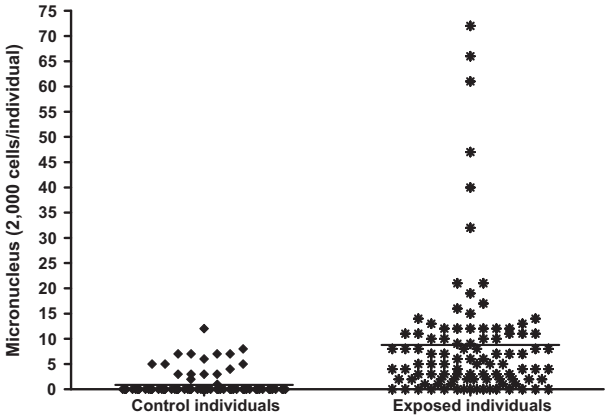

Fig. 1. Scatterplot comparing MN frequencies (BMCyt assay) of control individuals and exposed individuals. Horizontal lines represent the group mean MN frequencies.

The results obtained for MN frequency in exfoliated buccal cells showed that the values in the exposed group to coal mine residues are significantly higher compared with the control group, which were evaluated by Mann–Whitney *U*-test ($P < 0.001$). In addition, Table 1 lists additional markers of nuclear abnormalities in exfoliated buccal cells: nuclear buds, karyorrhectic and karyolytic cells. The mean values of the exposed group of these biomarkers were statistically significant compared to control group ($P < 0.05$).

The Spearman correlation coefficient of MN frequency with respect to age for the exposed ($P=0.7159$; $r=-0.03685$) *versus* control group ($P=0.1475$; $r=0.5272$) were not significant ($P > 0.05$). A high inter-individual variation between MN frequencies was only observed in the exposed group and ranged from 0 percent to 3.6 percent (0–72 MN/2000 cells) (Fig. 1). Fig. 2 shows the scatterplot comparing MN frequencies of the control group and exposed group divided by mining area activities. However, there were no significant correlations between the MN frequency and the service time for the different exposed groups ($P > 0.05$): extracted coal transport ($P=0.9116$; $r=-0.0500$); equipment field maintenance ($P=0.1618$; $r=0.5126$); coal stripping ($P=0.9359$; $r=-0.02112$); and coal embarking ($P=0.0760$; $r=-0.6360$). The MN data in buccal cells obtained in this study were compared with MN data in lymphocytes obtained in our previous study (León-Mejía et al., 2011). Fig. 3 shows a significant and positive correlation between MN frequency in lymphocytes and buccal cells of control and exposed individuals ($P < 0.001$; $r=0.573$).

The chemical elements present in the samples determinate by the PIXE method are presented in Table 2. The organic matrix of the blood (the organic composition of the sample) was 72.50 percent carbon, 7.50 percent oxygen, 13.50 percent nitrogen and 6.50 percent fluorine. There was no individual differences in the ppm concentrations of metals in the blood of workers measured by PIXE, in relation to the function performed in coal mining area as assessed by Kruskal–Wallis ANOVA and Dunn's post-test. Thus, for the evaluation of different correlations with regards to chemical present in blood samples, the whole study population of the exposed individuals was considered as the "exposed group". In the analysis of the difference between exposed and control group, the exposed group showed significantly higher ppm levels of aluminum (Al) and silicon (Si) using *t*-Student test with Welch correction ($P < 0.05$). The ppm amounts of metals showed no correlation with age or exposure time (Spearman correlation).

4. Discussion

Coal mining is an activity with a high potential for environmental pollution. In the case of exposure to coal mining residues, the studies that used biomarkers of biological effects, susceptibility and exposure as epidemiological tools are still scarce and most studies assessed underground mining activities (Agostini et al., 1996; Moriske et al., 1996; Santa Maria et al., 2007; Donbak et al., 2005). However, potential genotoxic effects caused by coal opencast mining activities on human health remain poorly explored.

In the present study, MN formation in exfoliated buccal cells of workers exposed to open coal mining was used as a biomarker for genotoxic exposure. This study did not show any effect of alcohol consumption, age and time of service on the MN frequency of the populations investigated. In concordance, Holland et al. (2008) cite that most occupational studies conducted with the buccal micronucleus cytome assay do not find a statistically significant influence of age and lifestyles in the MN frequency of study populations.

When we compared the MN frequencies in the group exposed to coal mining residues we observed a significantly higher frequency compared to the matched control group. No significant difference was observed in the extent of MN formation among the four different mining activities (transport of extracted coal, equipment field maintenance, coal stripping, and coal embarking). This observation indicates that the workers did show a genotoxic response to a complex mixture independent of the working area. Several individuals in the exposed group showed a higher MN frequency and high inter-individual variability. There was no clear difference in the exposed subgroups of the different working areas; therefore it can be assumed that there was no specific factor that would induce a particular high MN frequency. In a recent study using lymphocytes from coal mine workers from

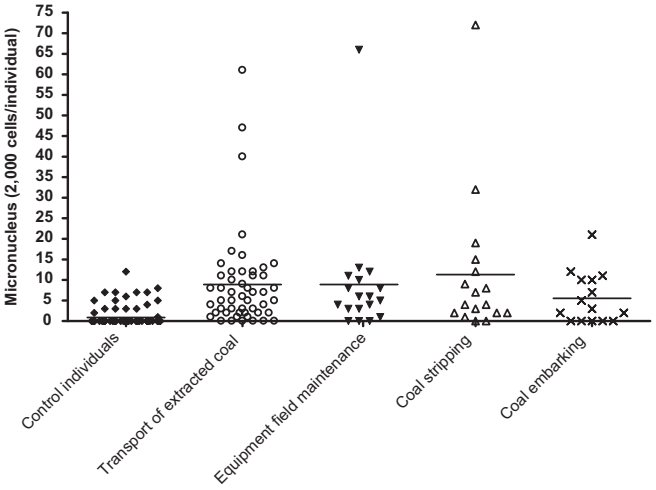

Fig. 2. Scatterplot comparing MN frequencies (BMCyt assay) of the control group and exposed group divided by mining area activities. Horizontal lines represent the group mean MN frequencies.

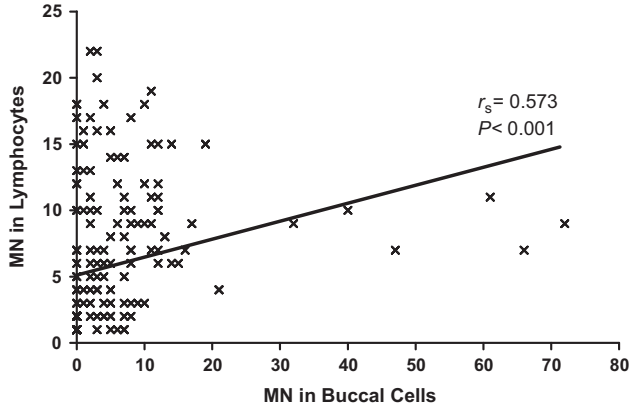

Fig. 3. Nonparametric Spearman correlation analysis between MN frequency in lymphocytes and buccal cells of control and exposed individuals (n=200).

Guajira-Colombia, we found comparable results with regards to occupational hazard effects using the Comet assay and MN test in lymphocytes (León-Mejía et al., 2011). These previous results demonstrated that the group exposed to coal mining residuals exhibited a significantly higher extent of DNA damage in peripheral lymphocytes in the Comet assay. In the exposed group, MN frequency was 2.9-fold and DNA damage index was 6.6-fold higher compared to the control group (León-Mejía et al., 2011), in this study we observed that MN frequency in buccal cells was 8.8-fold higher than control group. While the comet assay detects primary DNA damage with high sensitivity (Collins et al., 2008), the assessment of the MN frequency in isolated lymphocytes has become a reliable biomarker of chromosome breakage and/or whole chromosome loss (Fenech et al., 2003; Fenech, 2006). It has been demonstrated that high frequencies of MN in peripheral blood lymphocytes are predictive of cancer risk and that high levels of MN formation are associated with early events in carcinogenesis (Bonassi et al., 2007; Kirsch-Volders et al., 2014).

In our previous study (León-Mejía et al., 2011), MN frequencies were analyzed in lymphocytes from the same sample groups as those used in the current study, and these data were compared with data on MN in buccal cells from the current study. There was a significant and positive correlation between MN frequencies in the lymphocytes and buccal cells of the control and exposed individuals ($P<0.001$; r=0.573; Fig. 3). Similarly Ceppi et al.

Table 2
Concentration of inorganic elements in the blood samples (ppm) of the control group and exposed group divided by mining area activities by PIXE method (mean ± standard deviation).

Groups	Inorganic elements (ppm)												
	Na	Mg	Al	Si	P	S	Cl	K	Ca	Fe	Cu	Zn	Br	Rb
Control (n=100)	8942 ± 1657	209 ± 81	109 ± 50	44 ± 30	1629 ± 284	5717 ± 892	14,927 ± 2623	9285 ± 1481	312 ± 116	2945 ± 431	5 ± 2	39 ± 10	18 ± 9	17 ± 9
Exposed (n=100)	8049 ± 2019	210 ± 170	**127 ± 60***	**81 ± 95***	1529 ± 422	5396 ± 1051	13,572 ± 3366	8731 ± 2109	292 ± 106	2777 ± 538	5 ± 2	37 ± 10	17 ± 8	19 ± 11
Exposed per mining area														
Transport of extracted coal (n=50)	8042 ± 1900	219 ± 202	117 ± 63	71 ± 91	1525 ± 467	5401 ± 878	13,531 ± 2794	8675 ± 1786	291 ± 120	2783 ± 482	5 ± 2	37 ± 9	17 ± 8	18 ± 11
Equipment field maintenance (n=18)	8450 ± 2235	172 ± 89	150 ± 61	97 ± 123	1517 ± 360	5354 ± 1491	14,473 ± 3834	9001 ± 2315	298 ± 103	2854 ± 704	5 ± 2	37 ± 13	17 ± 7	18 ± 12
Coal stripping (n=17)	7855 ± 2398	205 ± 121	127 ± 46	91 ± 65	1526 ± 474	5388 ± 1234	13,003 ± 3916	8524 ± 2544	276 ± 81	2775 ± 635	4 ± 2	34 ± 11	15 ± 7	19 ± 9
Coal embarking (n=15)	7788 ± 1784	232 ± 183	133 ± 63	73 ± 101	1565 ± 290	5443 ± 779	13,216 ± 3973	8811 ± 2499	306 ± 91	2658 ± 363	5 ± 2	38 ± 8	18 ± 6	21 ± 10

* Significant increase in relation to the control group at $P<0.05$; Student's t test (Welch correction).

(2010) prepared a compilation of 19 studies which measured the MN frequency in buccal cells and lymphocytes showed a high correlation between both tissues, revealing that the MN evaluation in buccal cells has a similar potential to demonstrate the effects of exposure to genotoxic agents. The formation of micronuclei in both lymphocytes and epithelial cells has been proposed as a useful biomarker to assess the cytogenetic damage in biomonitoring studies (Diler and Celik, 2011; Rohr et al., 2013b; Kirsch-Volders et al., 2014). The formation of micronuclei in both lymphocytes and epithelial cells has been proposed as a useful biomarker to assess the damage cytogenetic in biomonitoring studies (Diler and Celik, 2011).

Our results further support that the mechanism of MN formation in buccal exfoliated cells is consistent with the model proposed for lymphocytes (recently reviewed by Ceppi et al. (2010)).

Besides the formation of MN, cell death indicators (karyorrhectic and karyolytic cells) and other nuclear anomalies such as nuclear buds (indicative of gene amplification) (Diler and Celik, 2011) were also evaluated in buccal cells. The results showed that the number of karyorrhectic and karyolytic cells were significantly higher in the exposed group compared with the control group. These markers of genetic damage found in our study suggest that these events could be a consequence of exposure to some genotoxic agents related to coal mining residues forming a complex mixture of agents present at low concentrations can interact additively or synergistically (Kalantzi et al., 2004), similar to what had been demonstrated by Rohr et al. (2013a, 2013b).

The main route of exposure of coal mine workers to potentially hazardous coal residues is by inhalation of particles. Today it is known that chronic inhalation of this cocktail (which may contain a mixture of substances such as inorganic elements and PAH) can produce pulmonary disorders (Schins and Borm, 1999; Beckman and Ames, 1997; Cooke et al., 2003). Some characteristics of coal from "El Cerrejon" are moisture (~10 percent), volatile (~30 percent), ash (~8 percent), sulfur (~1 percent), carbon (70 percent), hydrogen (~6 percent), oxygen (~5 percent), nitrogen (~1 percent) and different metals (ETSU and Department of Trade and Industry, 2000). In our study we included the assessment of several elements (Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb). The elements assessed in peripheral blood samples showed no difference when comparing the four mining area activities and no correlation with age or time of service was observed. In the analysis of the levels of the different elements in the study population we observed significantly higher amounts of silicon (Si) and aluminum (Al) in the exposed compared to the control group. In the composition of the Cerrejón-Guajira coal these elements are found in substantial quantities in the form of oxides (ETSU and Department of Trade and Industry, 2000), and presence of Al and Si in coal fly ash is recognized in coal fly ash (Prahalad et al., 2000). The abundance of different mineral elements in Cerrejón coal determined by Scanning Electron Microscopy Computer-Controlled shows that more than 80 percent of weight of the mineral material is composed of clay and quartz minerals (aluminum silicate, aluminum silicate, and silica). Analysis of the product reveals that the combustion ashes are formed mainly of aluminum silicates, iron oxide and quartz particles (Irons and Quick, 2000). It is known that inhalation of particulate material typically contains high levels of Al. Experimental studies indicate that the presence of excessive Al is associated with inflammatory processes in the lung which can trigger respiratory diseases (Clarke et al., 2000; Wagner et al., 2007) and carcinogenic processes (Spinelli et al., 2006; Exley et al., 2007; Neumann et al., 2011). The interaction of inorganic elements with living matter is complex, but it is possible that common mechanisms for the majority of inorganic compounds include oxidative stress, DNA repair modulation and disturbance of signal transduction pathway (Beyersmann and Hartwig, 2008).

PAH are also associated with the generation of oxidative stress. The spontaneous combustion of coal is very common in centers of open mining storage systems, and is a major cause of the production of PAH. Many PAH have mutagenic and carcinogenic effects (Cherng et al., 1996; IARC, 1997; Da Silva et al., 2000). Exposure to PAH has been associated with increased DNA damage by cytokinesis-block micronucleus cytome and oxidative stress in occupational exposed populations (Duan et al., 2009, Guo et al., 2014). Several studies have showed buccal MN induction in exposed populations (Giri et al., 2012, Karahalil et al., 1999) and *in vitro* studies suggest that PAH-quinones induce genotoxic effects by modulating the metabolic machinery inside the cells by a combined effect of oxidative stress (Gurbani et al., 2013, Ekstrand-Hammarstrom et al., 2013). Mixtures of DNA-reactive procarcinogens compounds such as PAH at environmentally relevant low-dose concentrations give rise to markedly elevated DNA damage (Hewitt et al., 2007). One of the proposed mechanisms of generation of DNA damage by exposure to PAH is associated with oxidation–reduction processes occurring during the metabolism of these compounds, which result in the formation of quinones. These quinones can undergo redox cycling and produce reactive oxygen species (ROS) (Singh et al., 2007). Another way of ROS generation by exposure to coal mine residues is related to the inhalation of coal dust particles which triggers an inflammatory cell response in macrophages and lung epithelial cells producing large amounts of ROS and cytokines. There is evidence of oxidative damage in coal mining workers, as higher levels of SOD (superoxide dismutase) in individuals exposed to coal (due to an enzymatic response) comparing with non-exposed individuals (Rohr et al., 2013a, 2013b). ROS may also be generated independently of the cellular pathway due to the intrinsic chemical properties of coal dust such as iron content and the radicals on the surface (Schins and Borm, 1999). It is known that ROS are capable of causing oxidative damage to DNA such as single strand breaks and base and nucleotide modifications, particularly in guanosine. The oxidative modifications induce a broad response in the repair characterized by excision of modified bases and nucleotides (Bennett, 2001; Klaunig et al., 2011).

5. Conclusions

In summary, increased levels of micronuclei in the BMCyt assay were observed in coal mining workers. The data of the present study are in agreement with the results of a previous study assessing DNA damage in lymphocytes using the comet assay and MN assay (León-Mejía et al., 2011). The increased MN frequencies observed in the mine workers may be a consequence of oxidative damage resulting from their exposure to coal residues mixtures, including inorganic elements, as Al and Si. However, there are several additional compounds that are released during the processes of exploration and extraction of coal and therefore, due to the complex mixture, it is difficult to relate the genotoxic effects found to the actions of a single compound. Therefore, our study demonstrates that buccal cells present a suitable and non-invasive source to investigate biomarkers of genetic damage in exposed individuals.

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