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Genotoxicity through Exposure to Particulate Matter (PM_{10}) at two Sites in the Valle de Aburrá, Colombia

[1]Verónica Estrada-Vélez, [1]Lucelly López-López, [1]Lina María Martínez-Sánchez, [1]Camilo Andrés Agudelo-Vélez, [1]María de los Ángeles Rodriguez-Gázquez, [1]María Victoria Toro-Gómez, [1,2]Luz Yaneth Orozco-Jimenez, [1]Mónica Zuluaga-Quintero, [1]Johan Sebastián Lopera-Valle, [1]Ana Paulina Pamplona-Sierra, [1]Ana María Díaz-Zuluaga, [1]Natalia Vargas-Grisales, [1]Ana María Vargas-Gallego, [1]Jennifer Rojas-Londoño, [1]Felipe Hernández-Restrepo, [1]Andrea Ocampo-Patiño, [1]Sara Rojas-Jimenez and [1]Isabel Cristina Ortiz-Trujillo

[1]Grupo Biología de Sistemas, Escuela de Ciencias de la Salud, Facultad de Medicina, Universidad Pontificia Bolivariana, Circular 1ra #70-01, Medellín, Colombia
[2]Research Group on Environmental Management and Modeling, Street 67 #53-108, Universidad de Antioquia, Medellín, Colombia

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Corresponding Author:
Lina María Martínez-Sánchez
Grupo Biología de Sistemas, Escuela de Ciencias de la Salud, Facultad de Medicina, Universidad Pontificia Bolivariana, Circular 1ra #70-01, Medellín, Colombia
Email: linam.martinez@upb.edu.co

Abstract: To establish differences in the genotoxic effect of particulate matter (PM_{10}) in exposed individuals at two sites in the Valle de Aburrá (VA). This was a descriptive cross-sectional study of 2 groups of individuals exposed to 67.7 and 45 µg/m^3 of PM_{10} for a minimum of 8 hours/day. The frequency of Chromosomal Abnormalities (CAs) and the mitotic index were evaluated based on blood samples. The data were processed using SPSS version 18.0 and significant differences were established between the groups at $p<0.05$. One hundred eighteen individuals were evaluated: 56.8% at site 1 and 43.2% at site 2. The study population had an average age of 53 years. Significant differences were found between the numbers of CAs ($p = 0.003$) detected at the sample sites. Site 1 displayed a higher number of CAs than site 2, likely because site 1 is located in an area of the VA that is more exposed to environmental contamination. Furthermore, this study showed that there is a relationship between the level of particulate matter and an increase in a biomarker for CAs, establishing a possible health risk in this population, especially for those who remain there for long periods of time.

Keywords: Mutagenesis, Air Pollutants, DNA Damage, Genotoxicity, Chromosome Aberrations

Introduction

To evaluate outdoor air quality is of great importance since an average adult (70 kg) can inhale close to 20 m^3 of air per day (Berne *et al*., 1998), constantly exposing them to hundreds of particles suspended in the air (de Brito *et al*., 2013). Multiple genotoxic agents can be attached to these particles, which can have adverse effects on human health (Coronas *et al*., 2009).

Inhalable particulate matter in the air encompasses particles that are 2.5 to 10 µm in diameter, commonly referred to as $PM_{2,5}$ or PM_{10}. Such particles can be deposited in the trachea, bronchi and bronchioles and $PM_{2,5}$ can even reach the pulmonary alveoli. Both $PM_{2,5}$ or PM_{10} particles are associated with negative effects on human health and on the ecosystem in general (Suárez and Pérez, n.d.). Other types of contaminants can attach to particulate matter, such as organic mutagenic and carcinogenic compounds. These compounds include Polycyclic Aromatic Hydrocarbons (PAHs), benzene, toluene and toxic inorganic compounds, such as sulfates, nitrates, ammonia and heavy metals (Zuluaga *et al*., 2009).

PAHs are environmental contaminants that form during the incomplete combustion of organic matter (Landvik *et al*., 2007). Lighter PAHs are predominantly found in a gaseous state, while heavier PAHs (with four or more rings) are primarily absorbed in particulate matter (Caballero and Alvarado, 2006). PAHs can be incorporated into an organism through ingestion, inhalation or through skin absorption due to their low water solubility and higher solubility in lipids. PAHs accumulate in organisms and in organic matter and sediments and can remain there for long periods of time, ensuring their bioavailability (Crebelli *et al*., 1995). Subsequently, PAHs can be absorbed by cells, where they reach the nucleus, bind to DNA through direct or indirect action and cause DNA adducts (genotoxic damage) (Talaska *et al*., 1996).

Mutagens such as PAHs are compounds that can interact with and damage DNA, disrupting the molecular structure of genes and leading to mutations. Mutations are the primary causes of many diseases, especially those that occur during development and carcinogenesis. Cancer is a disease in which dynamic changes in the genome can lead to alterations in gene function and, eventually, malignancy (Vogelstein *et al*., 2013; Hanahan *et al*., 2000). For this reason, the World Health Organization (WHO, 2004) has established that short-term and low-level (less than 100 $\mu g/m^3$) exposure to Particulate Matter (PM) in the air is associated with adverse health effects.Despite knowing the presence of PAHs in the atmospheric particulate material, this work did not identify them because it was not part of the objectives.

Different studies in Colombia that have been performed by investigators at the University of Antioquia (Daniels *et al*., 2007), the University of Medellín and the CES (Echeverri and Maya, 2008) have shown that the incidence of lung cancer has increased markedly in the Valle de Aburrá (VA). Specifically, the rate was 11.8 cases per 100 million inhabitants in 1980, with this number rising to 20.6 in 2005. Furthermore, an extraordinary increase in the automobile fleet has occurred in recent years in the VA, with a parallel increase in mobile sources and rates of air pollution (REDAIRE, 2012). Therefore, this study sought to establish whether there are differences in the genotoxic effect of particulate matter (PM_{10}) on exposed individuals at two sites in the AV.

Materials and Methods

Study Type

Descriptive cross-sectional.

Study Area

Two sample sites (Table 1) were selected in the VA region, which is characterized by a high degree of industrial development and with unequivocal signs of increased population. The VA currently has approximately 4 million inhabitants, approximately 2 million motor vehicles (PRO-ANTIOQUIA and Medelli, 2013) and extensive industrial and commercial activity within a relatively small geographical region (Bedoya, 2008). As a variable of air quality and environmental risk factor, the average daily concentration was considered for five atmospheric pollutants PM_{10}, sulfur dioxide (SO_2), Nitrogen Oxides (NO_X), Carbon monoxide (CO), Ozone (O_3). The registration of concentrations was obtained for all pollutants, through the operation of an automatic monitoring station ECOTECH brand. The compound NOx and O_3 by chemiluminescence methodology, CO with non-dispersive infrared, PM_{10} with TEOM equipment and SO_2 with Fluorescence. Regarding the characterization of the particulate material, the low vol PQ200 equipment was used in monitoring time 24 h for a total of 30 samples. The project did not have a budget to perform mass spectrometry.

Study Population

Residents of one of the sample were adults who had lived in the area for at least 5 years and were present in the area at least 8 h daily, after accepting the informed consent. All of the participants responded to a survey to determine their sociodemographic status. Individuals with mental illness, smokers, those undergoing medical treatments, those exposed to X-rays or those with vaccinations in the past 3 months were excluded from the study.

Sampling and Cell Cultures

A volume of 5 mL venous blood was obtained from each individual at each of the selected sites. The blood samples were transported and processed within four hours, after which the protocol described by Sorsa and Autio (1994) was followed. The slides were prepared and stained with Giemsa solution (4%) following standard cytogenetic methods (Fatima *et al*., 2001).

Table 1: The characteristics of the sample sites

Sample Sites	PM_{10} 24 h $\mu g/m^3$	Characteristics
One	67.7	High vehicular traffic; frequent increase in the level of particles PM_{10} with respect to the annual reference value for Columbia (REDAIRE, 2012).
Two	45.0	Low vehicular traffic; PM_{10} levels less than the reference value for Columbia (REDAIRE, 2012).

Cytogenetic Analysis

No karyotype was performed. The detection of Chromosomal Abnormalities (CAs) was performed using 100 metaphase cells per individual. Five structural categories of CA were evaluated: Chromatid Breaks (B), chromosomal breaks (BB), dicentric chromosomes, ring chromosomes (DC/R) and Multiradial chromosomes (MR) (Sorsa and Autio, 1994).

Statistical Analysis

Normality tests were performed and it was determined that all data were normally distributed. Pearson χ^2, χ^2 with Yates correction and Student's t-tests were applied to evaluate the differences between the study variables and the different sites. The data were processed using the statistical program SPSS version 18.0. Significant differences were established between the groups with a p-value <0.05.

Previous work was supported by the Ethics Committee of Health Investigations of the Faculty of Medicine and was performed based on Resolution N° 08430 of the Ministry for Social Protection from 1993, which classified this investigation as low risk.

Results

Of the 118 individuals analyzed, 43.2% (51 individuals) belonged to the site 1 group and 56.8% (67 individuals) to the site 2 group. There were no significant differences between the sites with respect to sociodemographic variables (Table 2). In general terms, it was observed that the study population, without distinguishing between the sample sites, consisted of individuals of approximately 50 years of age who were predominantly homemakers (34.3% and 43.1% for sites 1 and 2, respectively).

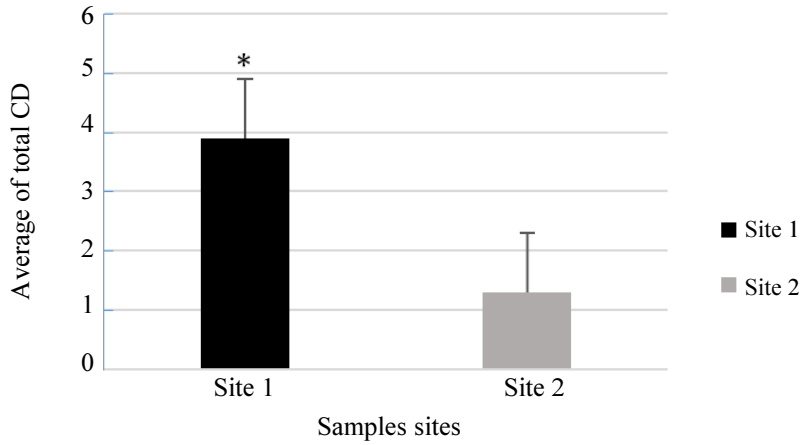

Fig. 1: Average number of total CAs in both populations exposed to particulate matter (PM_{10}).∗Statifically significant diference

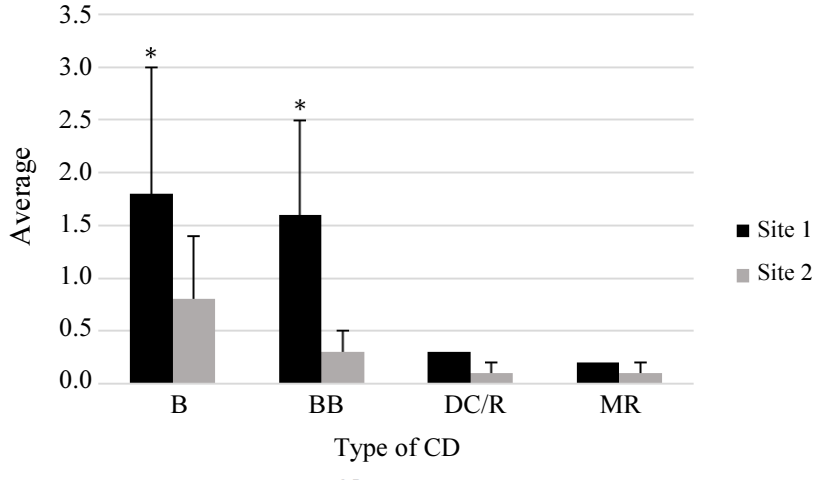

Fig. 2: Type of CAs in both populations exposed to particulate matter (PM_{10}). ∗Statifically significant diference

Cytogenetic Analysis: Significant differences in CAs at each sample site were found (Fig. 1) ($p<0.01$). The average number of CAs, considering all types, was higher at site 1 (Fig. 2). There was a significant difference in the number of chromatid (B) and chromosomal (BB) breaks in the site1 compared to site 2 ($p<0.05$). No differences are observed between the Dichromatic (DC) and Ring (R) chromosome numbers and the MR numbers between the sites ($p = 0.699$). A significant difference was found with respect to the mitotic index, with a slightly higher index at site 2 (2.7±0.5, $p = 0.014$). Figure 3 shows a normal chromosomal extension of human lymphocytes and Fig. 4 shows an extended chromosomal with chromosomal alterations of human lymphocytes found in the population of site 1.

Table 2: General characteristics of the study sample according to site

	Site		
Variable	One (n = 51)	Two (n = 67)	P-value
Female (%)[a]	59.7	81.6	0.012
Age (Mean ± SD)[b]	57.5±15.9	53.3±16.0	0.989
Marital status (%)[c]			0.385
Single	25.4	19.6	
Married	59.7	52.9	
Widowed	10.4	15.7	
Separated	4.5	9.8	
Common-law	0.0	2.0	
Education (%)[c]			0.253
None	7.5	0.0	
Elementary	37.3	39.2	
Secondary	28.4	37.3	
University	6.0	7.8	
Technical or technological	17.9	9.8	
Unknown, no response	3.0	5.9	
Occupation (%)[c]			0.114
Homemaker	34.3	43.1	
Employed	10.4	13.7	
Self-employed	25.4	11.8	
Pensioner	20.9	13.7	
Unemployed	4.5	2.0	
No response	4.5	15.7	
Hours at home (Mean ± SD)[b]	18.6±6.1	17.9±4.6	0.012
Steady work (%)[a]	35.8	31.1	0.613
Mitotic Index (Mean ± SD)[b]	2.5±0.5	2.7±0.5	0.014

[a]Pearson χ^2 without correction, [b]Student's t-test assuming that variances are equal, [c]χ^2 with Yates correction

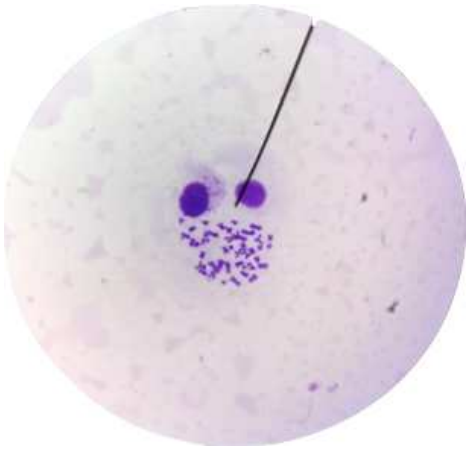

Fig. 3: Chromosomal extension of human lymphocytes exposed to Roswell Park Memorial Institute medium (RPMI) where no chromosomal alteration is evidenced

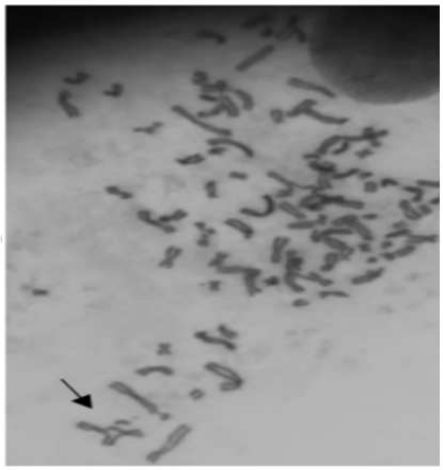

Fig. 4: Extended chromosomal human lymphocytes with evidence of chromosomal alteration (see arrow)

Discussion

Based on the results, it can be concluded that inhabitants of site 1, which has high levels of PM_{10} emissions, have elevated levels of DNA damage (Fig. 1). As the quantity of particulate matter increases, the quantity of genotoxic molecules attached to the particulate matter also increases (Claxton *et al*., 2004), an association that is reflected by the higher rate of CAs in individuals at site 1. These emissions have noxious effects on human health and the environment given that the aromatic compounds that are present in PM_{10} lead to chromosomal damage and breaks at non-cytotoxic doses.

In this study, a higher frequency of CA types B and BB were found in comparison to DC/R and MR at both sample sites (Fig. 2). This finding is because the type of CA depends on the lymphocytic cell cycle (Evans and Scott, 1963). For this reason, the present data suggest that the increased frequency of chromatid and chromosomal breaks could be caused by genotoxic molecules that accumulate in cells, causing type B and BB CAs when the cell goes S phase (Arboleda-moreno *et al*., 2004). The number of detectable effects of environmental contaminants on human health could be increased by implementing the use of biomarkers, such as CA, in environmental regulations (Zuluaga *et al*., 2009).

Conclusion

The present study showed stronger genotoxic effects in an area with higher contamination, demonstrating a relationship between the level of particulate matter and an increase in CA biomarkers. These data further establish a possible health risk factor for the population in the studied area. Nonetheless, it is necessary that additional studies (i) investigate the risks that such environmental contaminants can have, (ii) monitor air quality and (iii) perform biological analyses using CAs and other biomarkers. Such studies will provide good measures for determining the impact of contamination on the health of the population.

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Authors Contributions

Verónica Estrada-Vélez: Analysis and interpretation of results, statistical analysis, writing and correction of the article.

Lucelly López-López and María de los Ángeles Gómez-Gázquez: Statistical analysis, writing and correction of the article.

Lina María Martínez-Sánchez and Camilo Andrés Agudelo-Vélez: Preparation of reports, analysis and interpretation of results, statistical analysis, writing and correction of the article.

Luz Yaneth Orozco-Jiménez: Coordination in the taking of samples, preparation of reports, processing of samples (Laboratory work), analysis and interpretation of results, writing and correction of the article.

Mónica Zuluaga-Quintero: Preparation of reports, analysis and interpretation of results, writing and correction of the article.

Johan Sebastián Lopera-Valle, Felipe Hernández-Restrepo, Ana Paulina Pamplona-Sierra, Ana María Díaz-Zuluaga, Natalia Vargas-Grisales, Ana María Vargas-Gallego, Jennifer Rojas-Londoño, Andrea Ocampo-Patiño and Sara Rojas-Jiménez: Preparation of reports, processing of samples (Laboratory work), analysis and interpretation of results, writing and correction of the article.

Isabel Cristina Ortiz-Trujillo: Coordination in the taking of samples, coordination of all research, preparation of reports, analysis and interpretation of results, Statistical analysis, writing and correction of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

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