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Increased methylation of repetitive elements and DNA repair genes is associated with higher DNA oxidation in children in an urbanized, industrial environment

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ABSTRACT

DNA methylation in DNA repair genes participates in the DNA damage regulation. Particulate matter (PM), which has metals and polycyclic aromatic hydrocarbons (PAHs) adsorbed, among others has been linked to adverse health outcomes and may modify DNA methylation. To evaluate PM exposure impact on repetitive elements and gene-specific DNA methylation and DNA damage, we conducted a cross-sectional study in 150 schoolchildren (7–10 years old) from an urbanized, industrial area of the metropolitan area of Mexico City (MAMC), which frequently exhibits PM concentrations above safety standards. Methylation (5 mC) of long interspersed nuclear element-1 (LINE1) and DNA repair gene (*OGG1, APEX,* and *PARP1*) was assessed by pyrosequencing in peripheral mononuclear cells, DNA damage by comet assay and DNA oxidation by 8-OHdG content. PAH and metal contents in PM_{10} ($\leq$10 μm aerodynamic diameter) were determined by HPLC–MS and ICP-AES, respectively. Multiple regression analysis between DNA methylation, DNA damage, and PM_{10} exposure showed that PM_{10} was significantly associated with oxidative DNA damage; a 1% increase in 5 mC at all CpG sites in *PARP1* promoter was associated with a 35% increase in 8-OHdG, while a 1% increase at 1, 2, and 3 CpG sites resulted in 38, 9, and 56% increments, respectively. An increase of 10 pg/m^3 in benzo[b]fluoranthene content of PM_{10} was associated with a 6% increase in LINE1 methylation. Acenaphthene, indene [1,2,3-cd] pyrene, and pyrene concentrations correlated with higher dinucleotide methylation in *OGG1*, *APEX* and *PARP1* genes, respectively. Vanadium concentration correlated with increased methylation at selected *APEX* and *PARP1* CpG sites. DNA repair gene methylation was significantly correlated with DNA damage and with specific PM_{10}-associated PAHs and Vanadium. Data suggest that exposure to PM and its components are associated with differences in DNA methylation of repair genes in children, which may contribute to DNA damage.

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List of abbreviations

1-OHP	1-hydroxypyrene
5mC	5-methylcytosine
8-OHdG	8-hydroxy-2′-deoxyguanosine
APEX	Apurinic/apirymidinic endonuclease
CDC	Centers for Disease Control and Prevention
CpG	Dinucleotide cytosine-guanine
EC	Elemental carbon
IARC	International Agency for Research on Cancer
LINE1	Long interspersed nuclear element-1 (retrotransposable element)
MAMC	Metropolitan area of Mexico City
OC	Organic carbon
OGG1	8-oxoguanine DNA glycosylase
OTM	Olive tail moment;
PMNC	Peripheral mononuclear cells
PAHs	Polycyclic aromatic hydrocarbons
PARP1	Poly (ADP-ribose) polymerase 1
PM_{10}	Particulate matter with aerodynamic diameter $\leq 10\,\mu m$
ROS	Reactive oxygen species
t,t-MA	*trans,trans*-muconic acid

1. Introduction

Particulate air pollution (or particulate matter, PM) has been linked with numerous health problems, including cardiorespiratory diseases and cancer, among others. PM is a complex mixture of organic and inorganic compounds with a core of organic carbon (OC) and elemental carbon (EC) as well as adsorbed elements such as polycyclic aromatic hydrocarbons (PAHs) and metals [1,2]. The primary sources of PM in urban areas are industrial and vehicular exhausts [1]. Environmental exposure to PM can be extremely high in big cities, like the metropolitan area of Mexico City (MAMC). MAMC is one of the most populated and polluted cities in the world, and the annual and daily PM_{10} (PM with an aerodynamic diameter <10 μm) averages are often above Mexican and international safety standards [2,3]. An increase of 10 $\mu g/m^3$ of PM_{10} in the air was associated with a 1.83% increase in the number of deaths in MAMC [4]. Furthermore, PM_{10} collected in MAMC has demostrated mutagenic potential attributable to adsorbed PAHs [5,6] and *in vitro* exposure to PM_{10} extracts taken from MAMC was associated with genetic damage, apoptosis, and the production of proinflammatory mediators (tumor necrosis factor α, interleukin 6 and prostaglandin E2) [7]. A recent report showed that genetic damage-related diseases are the leading cause of mortality in children <14 years old in MAMC [8]. PM has recently been classified as a human carcinogen by the International Agency for Research on Cancer (IARC), as well as some PAHs and metals contained in them [9,10].

Exposure to PM causes oxidative stress and DNA damage [11]. Several redox metals, including iron (Fe), nickel (Ni), vanadium (V), and copper (Cu), released from PM can generate hydroxyl radicals via Fenton-type reactions, thereby promoting extensive oxidative damage in biomolecules. PAHs genotoxicity is driven by both, their metabolic activation to electrophiles that react with DNA and other molecules, and the reactive oxygen species (ROS) generated in these redox-cycling process [10]. Notably, an increase in cancer risk has been reported when the exposure to air pollution begins at an early age [12], making the evaluation of air pollution of great concern to the local community. Several reports have presented an association between exposure to $PM_{2.5}$ and DNA alterations [10], although $PM_{2.5}$ is an important constituent of the spatial average PM_{10} mass in MAMC [13], the study of the health effects of PM_{10} in an urbanized and industrial context with high PM_{10} concentrations, such as our study area is also necessary. Further, understanding the mechanisms underlying the health effects of PM exposure is critical to developing interventions to counteract it.

DNA damage and defects in DNA repair mechanisms are the first steps toward the development of many diseases [4]. Base excision repair (BER) is a major pathway involved in the repair of DNA oxidation. Eight-oxoguanine DNA glycosylase (OGG1), poly (ADP-ribose) polymerase (PARP1), and apurinic/apyrimidimic endonuclease 1 (APEX1) are enzymes involved in the first stages of the BER pathway [14]. Modifications to this DNA repair system contribute to an increase in DNA damage and to the accumulation of further mutations that are considered to be signs of early-stage disease [15]. Additionally, the expression of DNA repair enzymes is regulated by the methylation of their promoter regions [16].

DNA methylation involves the addition of a methyl group to carbon 5 in cytosine (5 methylcytosine; 5mC) by DNA methyltransferases (DNMTs) [17]. Methylation near gene promoters varies depending on cell type and, in general, high levels of methylation in gene promoters are associated with low or no gene transcription [18]. Similar to DNA damage accumulation, abnormalities in DNA methylation profiles are considered to be early signs of disease development [19]. Some reports in adult populations have shown a negative association between environmental PM_{10} exposure and methylation in intergenic long interspersed repetitive elements (LINE1) [17,20], as well as alterations in gene-specific methylation sites related to oxidative stress, cell cycle control, and DNA repair [20,21].

Alterations to the promoter region methylation of DNA repair genes may decrease the effectiveness of the repair system and contribute to increased DNA damage [22]. Few studies have evaluated changes in DNA methylation patterns in children exposed to air pollutants and there are no reports describing the impact of DNA methylation modifications on DNA repair genes in children exposed to high PM_{10}. In an effort to better understand how air pollution impacts DNA quality in children, we evaluated the methylation of DNA repair genes and the associated DNA damage in MAMC schoolchildren.

2. Materials and methods

2.1. Participants and sample collection

We conducted a cross-sectional study in November 2010 in a sample of 150 male and female schoolchildren (7–10 years old) recruited from the northern region of MAMC. Participants were randomly drawn from three schools that were selected to convenience in order to reflect the heterogeneity of air pollution in the study area. A sample size of 108 children was determined to be sufficient to detect a mean of 1% change in DNA methylation with 80% power based on a previous study conducted in adults [23]. The objectives and procedures involved in the study were explained to the parents or legal guardians of the participants, who signed the informed consent; child participants also provided their consent. All participants had living in the study area at least 6 months before participation in the study, and those with a cancer diagnosis or undergoing medical treatments were not included in the study. The study protocol was approved by the Institutional Ethics Committees at CINVESTAV-IPN and the National Institute of Public Health-Mexico in accordance with the ethical standards of the Declaration of Helsinki and its later amendments.

A structured questionnaire was to parents or legal guardians to collect detailed information regarding socio/demographic variables, lifestyle, past and recent health conditions, nutrition, and indoor and outdoor environmental exposures (e.g., biomass burn-