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EFFECTS OF AIR POLLUTION ON HUMAN HEALTH AND EPIGENETICS

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ABSTRACT

Air pollution is a significant threat to human genetics and health, as well as the environment. Components of air pollution, such as particulate matter (PM), black carbon (BC), ozone (O3), nitrogen oxides (NOx), and polyaromatic hydrocarbons (PAHs), have been linked to various health outcomes. Studies conducted on two different age groups, infants and the elderly, show that air pollution leads to decreased DNA methylation and other epigenetic marks, resulting in a weakened immune system and an increased incidence of autoimmune and respiratory diseases. During the first trimester of pregnancy, the effects of air pollution on DNA methylation are heightened, leading to fatal diseases, neonatal disorders, miscarriages, and infant mortality. This vulnerability to changes in DNA methylation and histone acetylation underscores the critical impact of air pollution on human health from the earliest stages of development.

KEYWORDS: Pollution, Methylation, Epigenetic, Immunity, Neonatal Disorders, Particulate Matter

INTRODUCTION

Air pollution is one of the major problems in today's world, affecting most cities, especially those in industrial zones. Almost 94 percent of the world's population lives in areas where the air quality does not meet the World Health Organization's guidelines, making these areas unsafe. Air pollution poses a significant threat to human health and epigenetics, altering epigenetic marks such as DNA methylation and histone acetylation present in the DNA of cells. Changes in DNA methylation can be observed throughout an individual's life, from the embryonic period to old age. DNA methylation is a critical process in understanding the effects of air pollution on gene activity and, consequently, on human health.

DNA methylation involves the attachment of methyl (CH3) groups to cytosine and sometimes adenine bases in an individual's DNA. These methyl groups lead to the formation of 5-methylcytosine (5-mC). In methylated genes, tRNA cannot bind to the promoter region, resulting in the gene being "switched off" and no protein synthesis occurring.

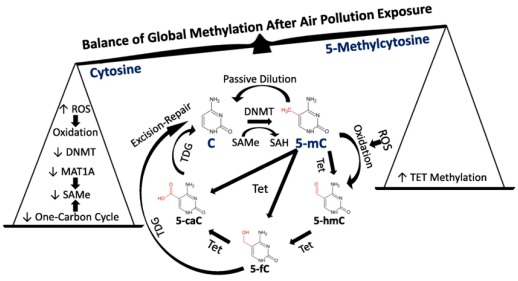

Figure 1: Rider & Carlsten (2019)

Changes in epigenetic markers can result in several health problems; the regulation of systems in the human body can be altered by these changes. For example, decreases in DNA methylation affect the immune system, making individuals more susceptible to diseases. One of the problems caused by air pollution is neonatal disorders. According to UNEP's air pollution note, 7% of deaths caused by air pollution are due to neonatal disorders. Air pollution is often associated with uncommon and risky factors that threaten babies' health, such as low birth weight and preterm birth. If babies are underdeveloped, this might lead to serious health problems like brain damage and inflammation, blood disorders, and jaundice, which can be fatal.

Similarly, changes in histone acetylation can lead to the misregulation of gene expression, triggering chronic diseases like cardiovascular diseases, asthma, and, in some cases, cancer.

Moreover, changes in epigenetic patterns can

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be inherited through generations. During meiosis, 99% of the epigenetic tags are removed from the DNA; however, those not removed may be passed through generations, creating a vulnerability to pollution-linked diseases.

Methodology

Experiment 1

This experiment aimed to analyze the impact of air pollution on DNA methylation and gene expression in placental tissue.

Participants: 100 healthy pregnant women were enrolled in prenatal clinics in Tehran, Iran. All participants were pregnant before the 14th week of pregnancy. Based on the calculated levels of particulate matter (PM) in the areas where the women resided, they were divided into two groups of 50 each. Participants experiencing changes such as relocation, employment changes, or constant traveling were excluded from the study.

Data Collection: At the time of birth, placental tissue was collected for DNA methylation and gene expression tests. Additionally, birth factors such as the newborn's sex, weight, height, head, and chest circumferences, and the level of neonatal care needed were recorded.

DNA Methylation Analysis: Genomic DNA was extracted from placental tissue using the standard method. In brief, DNA was extracted by the phenol method from homogenized placental tissues (Maghbooli et al., 2018). The DNA was hydrolyzed through several steps, and RNA was removed. RNA was initially removed by treating 50 µg of DNA in 300 µL of 1X Tris-EDTA buffer with RNase A (Fermentas Life Science, Cat No: EN0531) at a final concentration of 100 µg/mL and RNase T1 (Fermentas Life Science, Cat No: EN0541) at a final concentration of 2,000 units/mL. This treatment was carried out for 2 hours at 37°C, followed by ethanol precipitation until the global DNA methylation could be expressed as the percentage of 5-methyldeoxycytidine divided by the sum of 5-mC and deoxycytidine (dC): [5-mC/(5-mC + dC)]%.

Gene Expression Analysis: RNA was extracted from the placental tissue, and its quality was assessed through agarose gel electrophoresis. Gene expression was then analyzed using real-time PCR after cDNA synthesis. The presence of specific gene products was confirmed by using the melting curve method, which is an assessment of the dissociation characteristics of double-stranded DNA during heating. Six different primer sequences were used, and the mean value was obtained for further calculations.

Experiment 2

This experiment aimed to assess the impact of air pollution on DNA methylation and lung functionality in elderly men.

Participants: 776 elderly men (≥ 65 years of age) living in Boston, USA, were enrolled in the Normative Aging Study. Four clinical examinations were conducted between 1999 and 2009. During each visit, medication use, pulmonary disorders, and smoking history were recorded.

Data Collection: To measure DNA methylation, 7ml of blood samples were taken from each individual, and DNA was extracted. DNA methylation was quantitated using bisulfite-polymerase chain reaction and pyrosequencing (Tost & Gut 2007) near the promoter regions of a total of nine genes: Carnitine O-acetyltransferase *(CRAT)*, coagulation factor-3 *(F3)*, glucocorticoid receptor *(GCR)*, intercellular adhesion molecule *(ICAM1)*, interferon-gamma *(IFN-γ)*, interleukin-6 *(IL6)*, inducible nitric oxide synthase *(iNOS)*, 8-oxoguanine DNA glycosylase 1 *(OGG1)*, and toll-like receptor 2 *(TLR2)* (Lepeule et al., 2014). Methylation levels of nine specific genes related to cardiorespiratory health, inflammation, and oxidative stress mechanisms were measured using real-time PCR. Methylation analysis was repeated for each sample, and the results were averaged to minimize variability. Methylation levels were expressed as the percentage of 5-methyldeoxycytidine divided by the sum of 5-mC and deoxycytidine (dC): [5-mC/(5-mC + dC)]%.

Statistical Analysis: The effects of air pollution on lung functionality were formulated as: Yit=β0+ui+β1Air pollutantit+β2X2it+…+βpXpit+ϵitY_{it} = \beta_0 + u_i + \beta_1 \text{Air pollutant}_{it} + \beta_2 X_{2it} + \ldots + \beta_p X_{pit} + \epsilon_{it}Yit=β0+ui+β1Air pollutantit+β2X2it+…+βpXpit+ϵit

In this equation,

- YitY_{it}Yit represents the log-transformed lung function measurement for participant iii at visit ttt.
- β0\beta_0β0 is the intercept.
- uiu_iui is the random effect.
- β1\beta_1β1 is the effect of the air pollutant on lung function.
- X2itX_{2it}X2it to XpitX_{pit}Xpit are the covariates.
- Εit\epsilon_{it}ϵit represents the within-participant error.

This methodology provides a comprehensive approach to studying the effects of air pollution on human health and epigenetics, using both placental tissue analysis and longitudinal studies in elderly men.

RESULTS

Experiment 1

At the end of the experiment, 8 women were excluded due to relocation. Of the remaining 92 participants, 44 lived in non-polluted areas, while 48 lived in polluted areas. Post-birth data indicated that babies born in non-polluted areas were more developed in terms of height, weight, and circumferences. However, no correlation was found between birth outcomes and DNA methylation, as similar results were observed between the two regions.

The global DNA methylation percentages did not show significant differences between the two regions: non-polluted areas had a percentage of 2.44 (±0.86), and polluted areas had a percentage of 2.59 (±0.70). Notably, there was a significant correlation between DNA methylation and the amount of particulate matter in different trimesters. However, no significant correlation was found between gene expression and

the amount of particulate matter.

There were significant negative correlations between placental global DNA methylation and the gene expressions of SAMe and DNMT-1α genes (Maghbooli et al., 2018).

Experiment 2

The results of this experiment demonstrated that different particulate matters have varying effects on lung functionality. Black carbon (BC) and nitrogen dioxide (NO2) had the most significant estimated effects on lung function parameters (Lepeule et al., 2014).

Higher methylation levels were observed in participants exposed to higher levels of air pollution. Increased methylation led to the suppression of respiratory gene expression, resulting in poor lung and respiratory system function.

Genes such as GCR, F3, IL6, and TLR2 exhibited higher methylation, while the TLR2 gene had lower methylation. No correlation was found between the methylation of NO2, FEV1, LINE-1, and exposure to particulate matter.

The findings from both experiments highlight the impact of air pollution on DNA methylation and gene expression, which in turn affect human health. While no direct correlation was observed between DNA methylation and birth outcomes, particulate matter exposure significantly influenced DNA methylation patterns and gene expression related to respiratory health.

Discussion

The findings from this study clearly demonstrate that air pollution has significant effects on the epigenetics and health of individuals throughout their lives. Evidence from Experiment 1 (Maghbooli et al., 2018) and Experiment 2 (Lepeule et al., 2014) suggests that air pollution influences the amount of DNA methylation in an individual's DNA.

Impact on Early Development

In Experiment 1, it was observed that exposure to air pollution during the first trimester (zygotic stage) could influence DNA methylation and consequently gene expression. The first trimester is a critical stage of pregnancy when the baby's bodily systems are developing. Changes in epigenetic marks during this period can lead to serious health problems post-birth, including fatal diseases and infant mortality due to a weakened immune system and a lack of properly functioning proteins.

	Polluted (N = 48)	Non-polluted (N = 44)	P-value
PM 2.5 (μ/m^2)			
Whole pregnancy	37.12±.50	25.18±.68	0.0001
Trimester 1	30.99±0.86	20.43±.68	0.0001
Trimester 2	38.44±0.71	26.37±1.23	0.0001
Trimester 3	42.44±.74	29.04±1.11	0.0001
PM 10 (μ/m^2)			
Whole pregnancy	91.45±2.51	70.43±1.13	0.0001
Trimester 1	74.34±2.66	64.97±2.52	0.01
Trimester 2	94.88±3.35	74.06±1.65	0.0001
Trimester 3	104.89±2.61	72.13±1.33	0.0001

Numerical variables were expressed as the mean ± standard error (SE). PM2.5; fine particulate matter with a diameter 2.5 μm, PM 10; fine particulate matter with a diameter 10 μm.

https://doi.org/10.1371/journal.pone.0199772.t002

Figure 2: Maghbooli et al. (2018)

Impact on Elderly Individuals

Similarly, in Experiment 2, which focused on a completely different age group, similar results were observed. Exposure to air pollution disrupted the working rates of genes related to the respiratory system, causing poor lung function and leading to respiratory diseases such as asthma and lung cancer.

Broader Public Health Implications

Considering that 94% of the world's population is exposed to air pollution daily, it remains one of the world's most significant public health concerns. Environmental factors, including diet, exercise, and stress, also play a crucial role in epigenetic changes. However, air pollution, composed of various toxic gases and heavy metals, poses substantial health risks to those exposed.

Implications for Public Health

These findings underscore the need for stringent air quality regulations and public health interventions to mitigate the impact of air pollution on human health. Policymakers should consider

the long-term health effects of air pollution and take measures to reduce exposure, especially in vulnerable populations such as pregnant women and the elderly. Policymakers should enforce stricter air quality regulations to minimize exposure to harmful pollutants, particularly for vulnerable groups like pregnant women and the elderly. Additionally, policies should promote the use of renewable energy sources, enhance public transportation, and increase green spaces in urban areas to further improve air quality and protect public health, as several traffic-related air pollution (TRAP) components have been associated with changes in DNAm, including particulate matter (PM), black carbon (BC), ozone (O~3~), nitrogen oxides (NO~x~), and polyaromatic hydrocarbons (PAHs).

Public health campaigns should focus on raising awareness about the risks associated with air pollution and encouraging behaviors that reduce exposure. For instance, campaigns could educate the public about the benefits of using public transportation, carpooling, and reducing vehicle idling. They could also promote the use of air purifiers in homes, the importance of monitoring air quality indices, and the planting of trees to improve local air quality.

Hence, the study highlights the critical impact of air pollution on epigenetic changes and health outcomes. By understanding these effects, we can better address the challenges posed by air pollution and work towards improving public health through informed policy decisions and interventions.

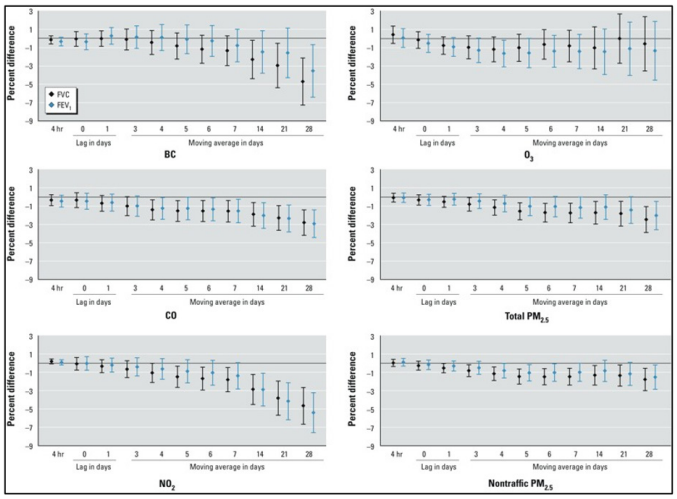

Figure 3: Lepeule et al. (2014b)

CONCLUSION

Exposure to air pollution at any stage of an individual's life can significantly alter their epigenetics and overall health. Evidence from Experiment 1 (Maghbooli, Zhila et al.) and Experiment 2 (Lepeule, Johanna et al.) suggests that air pollution can decrease or increase DNA methylation in undesirable ways. These changes in epigenetic markings pose a significant threat to human health, leading to a weaker immune system and increased vulnerability to diseases, particularly those related to environmental exposure.

The findings from Experiment 1 highlight that exposure to air pollution during the first trimester of pregnancy can influence DNA methylation, potentially leading to serious health problems post-birth. This underscores the critical importance of protecting pregnant women from air pollution to prevent adverse health outcomes for newborns.

Similarly, Experiment 2 demonstrates that air pollution exposure in the elderly can disrupt the functioning of genes related to the respiratory system, causing poor lung function and leading to chronic diseases such as asthma and lung cancer. This highlights the need for targeted interventions to protect vulnerable populations, including the elderly, from the harmful effects of air pollution.

These studies contribute to the growing body of evidence on the impact of air pollution on epigenetics and health, emphasizing the urgent need for public health policies to mitigate these effects. Future research should focus on identifying specific pollutants that cause the most harm and developing strategies to reduce exposure.

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