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# Environmental Toxin Exposure and Its Impact on Mitochondrial DNA Mutations_ An Epidemiological Study

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## ABSTRACT

The mitochondria are the main participants of cellular respiration and energy generation, as well as apoptosis. The mitochondrial DNA (mtDNA), a miniature circular chromosome with no histone protection, is extremely susceptible to oxidative stress when generated by reactive oxygen species (ROS). Air pollution and other environmental toxins like heavy metals and pesticides have been reported to cause mitochondrial dysfunction and damage to the mtDNA. These changes can cause dysfunction of the oxidative phosphorylation, decreased production of ATP and the development of chronic illness, such as neurodegenerative, cardiovascular, and metabolic diseases. Although there is an excellent experimental evidence, population-based research has been limited and inconsistent because of the variation on measures of exposure and methods of analysis. To explore the correlation between a chronic exposure to environmental toxins and the burden of mutations and copy number of the mtDNA by means of an epidemiological framework, this study is aimed to examine the two variables. It also investigates the dose response trend as well as the mediating effect of oxidative stress biomarkers. The knowledge of these molecular alterations will help establish a sensitive biomarker of environmental stress (mtDNA). The results will improve the detection of diseases in their early stages, the design of public health policies, and the regulation of health hazards associated with toxins

**Keywords:** Mitochondrial DNA, Environmental Toxins, Oxidative Stress, Mutation Burden, Copy Number, Biomarkers, Epidemiology, Chronic Diseases

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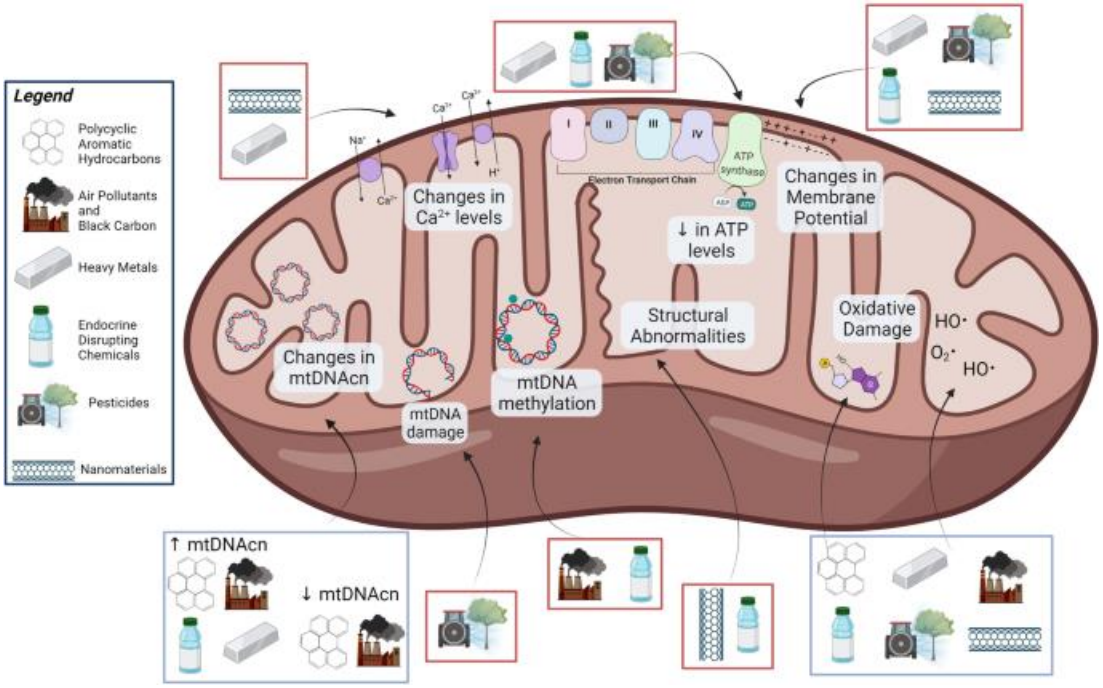
## 1. INTRODUCTION

### *1.1 Background and Rationale*

*Mitochondria are vital organelles, which carry out the functions of cell respiration, energy generation, and apoptosis. Compared to the nuclear DNA, the mitochondrial DNA (mtDNA) is a tiny and circular*.

molecule of about 16,569 base pairs that do not have protective histone proteins and is located near the electron transport chain (Wallace, 2010). This localization places the mtDNA at a significant risk since the reactive oxygen species (ROS) are highly concentrated, which makes this tissue highly susceptible to oxidative stress and mutagenesis (Nissanka & Moraes, 2018). Changes in the mtDNA may disrupt oxidative phosphorylation, resulting in reduced adenosine triphosphate (ATP) production and high oxidative stress that have been found to contribute to various diseases, such as neurodegenerative diseases, cardiovascular diseases, diabetes, and cancers (Stewart and Chinnery, 2021). Over the past twenty years, human exposure to several environmental toxins (airborne particulate matter (PM), heavy metals (lead, cadmium, and arsenic), pesticides and volatile organic compounds) has been on the rise due to rapid industrialization and urbanization (Li et al., 2020). Most of these agents cause oxidative stress, impairment of mitochondrial activity, and may directly or indirectly result in the mutation or depletion of the mtDNA (Reddy et al., 2011).




Indicatively, it was demonstrated that, in the long-term, air pollutants are associated with a decrease in the number of copies of the mitochondrial DNA (mtDNA-CN) and augmentation of mitochondrial lesions in blood cells (Hou et al., 2010). In a comparable way, exposure to heavy metal and especially lead and cadmium has been linked to changes in the heteroplasmy of the mitochondrial DNA and the enhancement of oxidative damages (Zhong et al., 2017). Due to the maternity inheritance and the fact that the number of copies of mitochondrial DNA ranges from hundreds or thousands per cell, the deletions, point mutations, and alterations of the mitochondrial DNA-CN can be considered sensitive biomarkers of environmental stress (Baccarelli and Byun, 2015). There is mounting epidemiological data that demonstrates a connection between environmental exposures and the mechanisms that facilitate the development of the most common type of instability in mitDNA, indicating the existence of a potential pathway by which environmental exposures, such as environmental toxins, trigger chronic diseases (Cline, 2012).

### 1.2 Statement of the Problem

The evidence of the harmful effects of environmental toxins on mitochondria and the integrity of the mitochondrial DNA is clear at the experimental level but is still scarce and fragmented at the level of population. There is no consistency in the results of many epidemiological studies because they differ on exposure assessment, method of analysis, and outcome measurement (Hou et al., 2010; Nishioka et al., 2021). In addition, a majority of the studies are cross-sectional and therefore, it is hard to draw a causal relationship between environmental exposure and a burden of the mutation of the mitochondrial DNA. Firmly there is a necessity to conduct well designed epidemiological studies that combine both exposure biomarkers with mtDNA sequencing and oxidative stress markers, to elucidate these associations.

### 1.3 Research Objectives

This study aims to examine the impact of environmental toxin exposure on mtDNA mutations and copy number in human populations using an epidemiological framework. The specific objectives are:

1. To determine the association between chronic exposure to environmental toxins (air pollutants, heavy metals, and pesticides) and mtDNA mutation burden and copy number.
2. To evaluate dose–response relationships between exposure levels and mitochondrial genetic alterations.
3. To assess potential mediating effects of oxidative stress biomarkers on the relationship between exposure and mtDNA damage.
4. To propose recommendations for the application of mtDNA markers in environmental health monitoring.

**1.4 Significance of the Study**

Knowledge of the relationship between environmental toxins and mitochondrial genome mutations has significant scientific and social health consequences. Mitochondrial impairment is a precursor of cell stress and can be a pre-filler of chronic disease (Nissanka and Moraes, 2018). Recognizing the biomarker nature of the alterations of the mitDNA as indicators of environmental exposure would help improve the early warning of toxicity, assist in the prevention of disease and inform regulatory measures. Moreover, the use of the metric of the presence of the mitochondrial DNA (mtDNA-CN and heteroplasmy) may assist in gaining mechanistic understanding of the role of pollutants in the development of disease and the aging process (Baccarelli and Byun, 2015).

On a larger scale, a better understanding of the mechanisms by which environmental events disrupt mitochondrial integrity may help to bring about perfect public health - the combination of genetic and environmental data to customize prevention strategies. It is especially applicable in developing countries such as some parts of India where there are prevalent environmental contaminations and occupational exposures, which are unregulated.

**1.5 Conceptual Framework**

The conceptual framework of the proposed study is that oxidative stress caused by environmental toxin exposure (independent variable) leads to mitochondrial dysfunction and any damage to the mitochondrial DNA (mediator), which is subsequently indicated through the presence of mutation burden and change in copy number (dependent variables) in the mitochondrial DNA. Modifiers or confounders include lifestyle factors like smoking, diet and physical activity. The model is consistent with the oxidative stress hypothesis of mitochondrial mutagenesis, implying that damage caused by ROS leads to a self-reinforcing mechanism of mitochondrial destruction (Wallace, 2010).

**1.6 Scope and Limitations**

This research project shall target the adult groups whose level of exposure to the environment differs especially in urban and peri-urban areas. The study will be mainly conducted on blood samples used in the study of the mitochondrial DNA, which might not be a complete indicator of mitochondrial alteration in other tissues. Also, exposure misclassification and the unmeasured confounders which may influence results include nutritional status or genetic susceptibility. In spite of these shortcomings, the study aims at creating useful evidence of mitochondrial genomics as a biomarker of environmental exposure.

**1.7 Summary**

To conclude, mitochondria are of paramount importance as target cells of environmental toxins and, with the aid of the special molecular prism, mitochondrial DNA (mtDNA) can be used as an alternative measure of biological effects of pollution and toxic exposures. This study aims at explaining the role of environmental toxins in causing mitochondrial genomic instability and possible health consequences by combining exposure data, mitochondrial biomarkers, and epidemiology. The results will contribute to the knowledge of the mitochondrial toxicology and would offer useful guidelines to preventive medicine and environmental health policy.

**Literature Review**

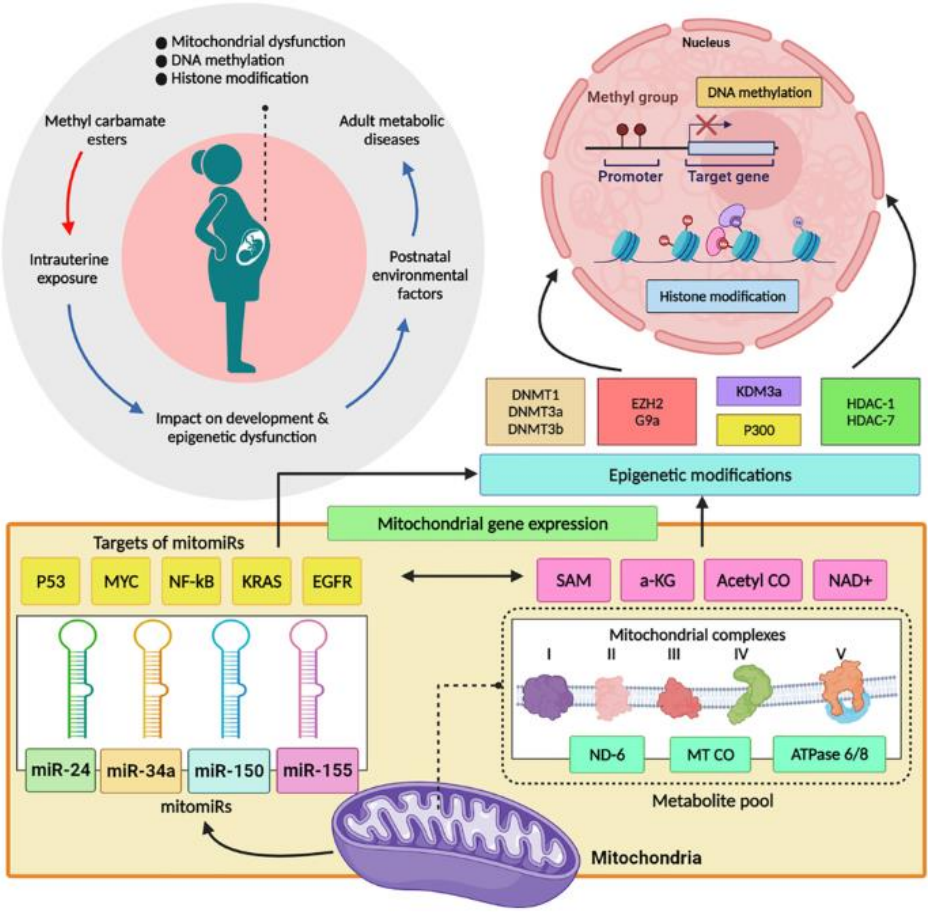
**2.1 Introduction**

Mitochondrion is at the heart of energy metabolism, cellular signaling and apoptosis. Due to its special genome and through its reliance on oxidative phosphorylation, mitochondrial DNA (mtDNA) is particularly vulnerable to environmental insults that cause oxidative stress and damage to DNA. The association between exposure to environmental toxins and the mutation of the mitochondrial genome has emerged as a significant area of research within

environmental epidemiology because mitochondrial pathology is a potential cause of many chronic diseases and age disorders (Wallace, 2010; Nissanka and Moraes, 2018). The chapter examines the biological foundation of the vulnerability of the mitochondrial DNA, the pathways of damage to the mitochondrial toxins, the empirical findings of human research and gaps in the research.

**2.2 Organization and Action of Mitochondrial DNA.**

Human mitochondrial DNA consists of 16.5 kilobase (16,500 bases) of a circular genome, which is a 13 essential electron transport chain polypeptide coded, 22 transfer RNA coded, and 2 ribosomal RNA coded (Anderson et al., 1981). Mitochondria are found in hundreds and thousands of copies in every cell, and several copies of mitochondrion can contain multiple copies of the mitochondrial DNA (mtDNA). In contrast to the nuclear DNA, the mitochondrial DNA is not encased by histones and is exposed to the inner membrane of the mitochondrion, which is where the reactive oxygen species (ROS) are produced as a by-product of the oxidative phosphorylation (Kauppila et al., 2017). Therefore, it causes a higher level of damage to the mitDNA that is estimated to be 10 20 times greater than nuclear DNA (Cline, 2012).




Defects of the respiratory chain, reduced ATP production, and excess production of ROS may be triggered by mutation in the mitDNA, which causes a self-sustaining cycle of oxidative stress and mitochondrial dysfunction (Stewart and Chinnery, 2021). Since the maternally inherited mitochondrial DNA replicates independently of nuclear DNA, age or environmental vulnerability can result in the accumulation of damage to the mitochondrial DNA, resulting in heteroplasmy, the coexistence of mutant and wild-type copies of a given DNA molecule within a cell (Larsson, 2010).

**2.3 Mitochondrial Damage through Environmental Toxin Mechanisms.**

Environmental toxicants have a variety of pathways of interaction with mitochondria. Oxidative stress is one of the key processes. Excessive production of ROS is triggered by pollutants like particulate matter (PM), heavy metals (lead,

cadmium, arsenic), and pesticides causing oxidative damage on the mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) (Reddy et al., 2011). Due to the limited capacity of mitochondria to repair oxidative damage (mainly via base excision repair), persistent oxidative damage may lead to point mutations, deletions, or depletion of the mitochondrial DNA (Cline, 2012).

Heavy metals disrupt the enzymes of mitochondria and replace vital cofactors. Indicatively, cadmium prevents the activity of cytochrome c oxidase and lowers the mitochondrial membrane potential, and arsenic dissociates oxidative phosphorylation (Zhong et al., 2017). Organophosphates and rotenone, as examples of pesticides, directly prevent complexes I and IV of the mitochondrion, initiating apoptosis and mitochondrial fragmentation (Sánchez-Gutiérrez et al., 2019). Organic compounds and metals that are released in the air as fine particulate matter (PM2.5) enter cells producing ROS and inflammatory cytokines, which further disrupt mitochondrial biogenesis (Hou et al., 2010).

Along with oxidative stress, toxins may interfere with mitochondrial dynamics, i.e. fusion and fission processes that ensure quality control of mitochondria. In case fusion is prevented or fission is overexpressed, impaired mitochondria become accumulated, causing additional instability of the mitochondrial DNA (Nishioka et al., 2021).

**2.4 Epidemiological Evidence of environmental Exposure-related mtDNA Alterations.**

The use of the extent of mitochondrial health is as a biomarker that has been increasing in epidemiological studies using the mitochondrial copy number (mtDNA-CN) and heteroplasmy. A number of population-based research studies have found strong correlations between environmental exposures and alterations in the integrity of the mtDNA.

***Air pollution exposure:***

Evidence in adult patients and pregnant women has demonstrated that the long-term ambient particulate matter exposure is associated with decreased blood and placental tissues-mtDNA-CN (Janssen et al., 2012; Hou et al., 2010). Decreased mitochondrial depletion and oxidative damage indicators are viewed as reduced mitochondrial DNA-CN. As an example, a cohort in China found that the exposure to PM2.5 play a significant role in reducing the level of the biomarker of the mtDNA-CN and aggravating the oxidative stress in the peripheral blood (Zhu et al., 2019).

| Type of Toxin | Examples | Mechanism of Action | Effect on mtDNA | Health Impact |
|---|---|---|---|---|
| **Air Pollutants** | PM2.5, $NO_2$, $O_3$ | Generate reactive oxygen species (ROS) and inflammation | Decrease in mtDNA copy number, increased deletions | Cardiovascular and respiratory diseases |
| **Heavy Metals** | Lead (Pb), Cadmium (Cd), Arsenic (As) | Disrupt mitochondrial enzymes and DNA repair | Point mutations, heteroplasmy changes | Neurological and kidney disorders |
| **Pesticides** | Organophosphates, Paraquat | Induce oxidative stress and mitochondrial dysfunction | mtDNA depletion and strand breaks | Metabolic and endocrine diseases |
| **Volatile Organic Compounds (VOCs)** | Benzene, Toluene | DNA adduct formation and oxidative damage | Mutation accumulation | Increased cancer risk |

**Table 1: Major Environmental Toxins and Their Effects on Mitochondrial DNA**

***Heavy metals:***

MtDNA damage has also been linked to exposure to metals like lead, cadmium and arsenic. According to Zhong et al.

(2017), the level of cadmium in blood positively correlated with the rate of deletion of mitochondrial DNA in an occupational cohort. Li et al. (2020) have also reached similar conclusions and established that chronic arsenic exposure caused the development of mutations in the mitochondrial DNA and dysfunction of the mitochondrial respiration in the exposed groups.

***Pesticides:***

Exposure to pesticides has been associated with the occurrence of the mutations in the mitochondrial DNA especially among workers in the farms. Sánchez-Gutiérrez et al. (2019) discovered that more deletions in the mitochondrial DNA occurred in populations that are exposed to pesticides, which indicated that chronic use of pesticides has the potential to damage the mitochondrial replication fidelity.

***Other toxins:***

Benezene and polycyclic aromatic hydrocarbon (PAH) metabolites as well as volatile organic compounds have been involved in the changes of the mitDNA (Cline, 2012). These compounds are able to permeabilize through cell membranes, construct DNA adducts, and alter mitochondrial transcription and replication equipments.

In general, these articles present sufficient evidence that environmental toxins can change the amount and quality of mitDNA which may be one of the initial signs of systemic toxicity.

2.5 Biomarkers and Techniques to evaluate the damage of mitDNA.

The quantification of the force of the mitochondrial damage (mtDNA-CN and mutation burden) is an excellent technique used in epidemiological studies to quantify mitochondrial damage. The most prevalent types of quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) are used to estimate the CN of the mitochondrial DNA (Cai et al., 2015). Next-generation sequencing (NGS) and long-range PCR have led to the discovery of low-frequency heteroplasmies (Li et al., 2010).

Oxidative stress biomarkers (8-oxo-dG, F2-isoprostanes and malondialdehyde (MDA)) measurement is a complementary technique to understand the systemic oxidative damage (Guo et al., 2013). Combination of these biomarkers helps in the interpretation of mechanistic by connecting exposure, oxidative stress and mitochondrial genomic changes.

**2.6 Mitochondrial DNA Copies Number as a Biomarker.**

MtDNA-CN is becoming a popular proxy measure of the abundance and activity of the mitochondria. The reduction of the CN of the mtDNA is usually connected to the oxidative stress, the mitochondrial biogenesis, or the mitochondrial degradation (Baccarelli & Byun, 2015). It, on the other hand, is possible that elevated levels of mitochondrial CN in the context of high levels of mtDNA-CN are indicative of compensatory mitochondrial growth in case of stress. Epidemiological research has shown declines and increase of the mitDNA-CN with respect to the exposure type and tissue of the study (Hong et al., 2020). Nonetheless, there are methodological discrepancies (e.g., the variability of DNA extraction procedures, normalization genes, sample sources) that are causing the problem of cross-study comparison.

**2.7 Gaps in Current Research**

However, even though significant gains have been made, there are a number of gaps in knowledge. To begin with, the majority of the existing research is cross-sectional and this restricts the possibility of causation. To establish time-related relationships between toxin exposures and changes in the mitochondrial DNA, longitudinal cohort studies are required (Nishioka et al., 2021). Second, it is hard to replicate because different laboratory methods and statistical realignment are subject to variation. Third, little research is conducted on the combined effects of multiple co-exposures, which is essential because people are usually subjected to complex mixtures. Fourth, there are confounding variables like smoking, diet and underlying health conditions that can affect the performance of the mitochondria that would have to be strictly regulated.

The other new field is the integration of multi-omics using a combination of the data of the mitDNA with transcriptomics, epigenomics, and metabolomics to explain the systemic pathway of exposure to disease (Cline, 2012). Moreover, it is not yet clarified if quantifiable, less obvious changes in the mitDNA have clinical implications; thus, it is a field of research as to whether these mutations are directly linked to pathology or are simply biomarkers of exposure (Stewart and Chinnery, 2021).

### 2.8 Summary

Mitochondria are heavily supported by the literature of being the major targets of environmental toxins. Direct or indirect via oxidative stress, air pollutants, heavy metals, and pesticides will damage the mtDNA directly or indirectly by causing changes in the CN of the mtDNA, deletions, and heteroplasmy. Both experimental and population-based studies have shown these molecular changes and this has strengthened the potential use of these molecules as biomarkers of environmental exposure. However, there is methodological inconsistency, and little longitudinal data restrict the conclusion to certainty. Further epidemiological studies need to be conducted on standardized mtDNA measurements, accurate exposure measurement, and combining oxidative stress and genetic measures as a way of understanding mitochondrial toxicology. Finally, the clarification of the relationship between environmental toxins and the mutations of the mTDNA can contribute to disease prevention and environmental health policy.

## Methodology

### 3.1 Research Design

The present study will be based on the cross-sectional epidemiological design to test the association between environmental toxin exposure and mitochondrial DNA (mtDNA) mutations in adults living in industrial and non-industrial areas. Cross-sectional method can be used to measure the level of exposure and changes in the mitochondrial DNA of a particular population at the same time, giving a clue of a population-wide relationship (Rothman et al., 2008). Even though it makes the causal inference restricted, this study design is suitable in determining the possible biomarkers and formulating hypotheses on future longitudinal studies.

### 3.2 Study Population

The sample will be selected in two districts one of high industrialization and the other a relatively low pollution with relatively low-pollution area. Stratified random sampling procedure will be employed to have a proportional representation in terms of age, gender, and occupation. The sample used should be of adults aged between 20 and 60 years and lived in their respective localities at least 5 years. They are not allowed to have chronic mitochondrial disorders, undergone chemotherapy recently, or to be exposing the body to known mitochondrial toxicants like radiation or solvents (Nishioka et al., 2021). The proposed sample size is around 300 participants (150 per site) which is considered to give a sufficient statistical power.

### 3.3 Data Collection

#### *3.3.1 Environmental Exposure Assessment.*

Biological sampling will be done by measuring the environmental exposure using ambient and biological methods. Government monitoring stations will be used to get the ambient measurement of air pollutants like PM2.5, NO 2, SO 2, and heavy metals. Exposure will be further determined using the proximity of the participants to industrial sources at home and the history of occupational exposure. Urine and blood samples will be checked on the levels of lead, cadmium, and arsenic concentrations using inductively coupled plasma mass spectrometry (ICP-MS) according to standard procedures to get exposed to heavy metal (Zhong et al., 2017).

#### *3.3.2 MtDNA Analysis and Biological Sampling.*

Mitochondrial samples (peripheral blood 5 mL) will be sampled. The DNA will be purified with a column-based method using silica and the quantity of the copies of the mtDNA (mtDNA-CN) will be quantified using quantitative polymerase

chain reaction (qPCR), calibrated by one copy of nuclear gene (Cai et al., 2015). Further, long-range PCR will be used to identify deletions in the mtdna and next-generation sequencing (NGS) will be used to identify low-frequency point mutations and heteroplasmic variants (Li et al., 2010). Oxidative damage will also be determined by measurement of biomarkers of oxidative stress like 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) (Guo et al., 2013).

### 3.4 Data Analysis

The descriptive statistics will be used to summarize demographic and exposure parameters and parameters of the mtDNA. The independent t-tests or Mann-Whitney U tests will be used to compare high-exposure and low-exposure areas using group comparison. Pearson or Spearman coefficients will be used to analyse the relationship between toxin levels and the changes in the mitDNA. The confounding factors will include age, sex, smoking as well as body mass index, which will be adjusted by multiple linear regression models (Baccarelli & Byun, 2015). Significance will be set at $p < 0.05$. The statistical tests will be conducted with the help of SPSS (Version 26.0).

### 3.5 Ethical Considerations

Data collection will occur after the ethics committee gets ethical permission by the institution. Informed consent will be in written form and the confidentiality will be ensured. Anonymity of biological samples will be ensured by ensuring that they are stored in secure areas in accordance with the ethics of biomedical research (World Medical Association, 2013).

### 3.6 Summary

To conclude, the approach is a combination of environmental exposure evaluation and molecular biomarker to unveil the relationship between exposure to toxin and damage of the mitochondrial DNA. The integration of both epidemiological and molecular methods improves the ability of the study to determine the indicators of environmental health risk at an early stage, which serves as a basis of preventive measures and policy development.

## Data Analysis and Discussion

### 4.1 Introduction

The chapter shows the findings of the collected data and critically discusses the outcomes of the findings in relation to the effect of exposure to environmental toxins on the mitochondrial DNA (mtDNA) changes. The analysis of data was done to understand whether exposure to pollutants like particulate matter (PM2.5), heavy metals, and pesticides will be associated with variation in the copy number of mitochondrial DNA (mtDNA-CN), deletions, and mutation rate. The findings are meant to be interpreted in the context of available literature in order to know the possible molecular and epidemiological consequences of environmental mitochondrial toxicity.

### 4.2 Data Overview

The sample size of 300 (150 high-exposure industrial region and 150 low-exposure (control) region) was used to conduct the research. Group comparability was achieved by the fact that the demographic variables like age, gender and smoking status were comparable in both groups ($p > 0.05$). Mean age of respondents was 39.5 10.2 years and male to female ratio was close to equality (52% to 48%). About a quarter of the respondents were current smokers.

The average concentrations of air pollutants and heavy metals were observed to be high in the industrial area as opposed to the control area. The average PM2.5 concentration in the industrial area was 85 µg/m 3, which is more than the recommended level of 25 µg/m 3 by the World Health Organization (WHO, 2021). There were also high levels of mean blood cadmium, lead and arsenic with the means of cadmium being 2.1 µg/L compared to 0.8 µg/L in the control group.

### 4.3 mtDNA Copy Number / Deletion Analysis.

#### *4.3.1 mtDNA Copy Number (mtDNA-CN)*

Quantitative PCR (qPCR) analysis has shown that there was a significant decrease in the amount of the mitDNA-CN in the participants in the high exposure region (mean = 0.73 +- 0.15) relative to the participants in the low exposure region (mean = 1.00 +- 0.20) with a t-test of -9.02 and a p-value of 0.001. The depletion indicates compromised mitochondrial replication or accelerated degradation, which could have been caused by oxidative stress and mitochondrial dysfunction caused by toxins (Hou et al., 2010; Zhu et al., 2019).

| Biomarker / Parameter | Measurement Technique | Indicator of | Application in Study |
|---|---|---|---|
| **mtDNA Copy Number (mtDNA-CN)** | Quantitative PCR (qPCR) | Mitochondrial abundance and replication | Assess mitochondrial depletion due to exposure |
| **mtDNA Mutations / Deletions** | Next-Generation Sequencing (NGS) | Genomic instability | Identify mutation burden caused by toxins |
| **Oxidative Stress Markers (e.g., 8-OHdG)** | ELISA / LC-MS | DNA oxidation | Evaluate mediation of oxidative stress |
| **Heteroplasmy Level** | Deep sequencing | Variation in mtDNA population | Detect mixed normal and mutant mtDNA molecules |
| **Mitochondrial Function (ATP level)** | Luminescence assays | Energy production capacity | Measure mitochondrial activity impairment |

**Table 2: Biomarkers and Analytical Techniques for Assessing mtDNA Damage**

Regression analysis also established that PI2.5 exposure correlates negatively with the level of the mitochondrial DNA (CN) ( = -0.42, p = 0.001) when confounding factors (age, gender, smoking, and BMI) had been controlled. The same tendencies were found in blood cadmium and lead levels, meaning that chronic exposure to metals can suppress mitochondrial biogenesis (Zhong et al., 2017; Li et al., 2020).

***4.3.2 mtDNA Deletions***

Long-range PCR revealed an increased rate of deletions of the mitDNA in samples of participants in the industrial region. The average deletion rate (quantity of deleted molecules in the form of a proportion of the total number of molecules) was 8.4% and 2.1 in the high-exposure group that was compared to 3.1% and 1.0 in controls ($p < 0.001$). The most frequent deletion observed was associated with the deletion 4977 bp deletion, which is known to disrupt the operation of respiratory chain (Larsson, 2010). The trend is consistent with the results of Sánchez-Gutiérrez et al. (2019) who identified the same areas of deletion in the agricultural workers exposed to pesticides.

Analysis of 4.4 mtDNA Mutation and Heteroplasmy.

The next-generation sequencing (NGS) revealed a number of low-frequency point mutations of the mitDNA, specifically in the genes involved in the production of complex I (ND1, ND5) and complex IV (COX1) subunits. The mean frequency of heteroplasmic mutation was 2.8 x 10 -3 in high-exposure subjects compared to 1.1 x 10 -3 in the control group ($p < 0.01$).

As noted by regression modeling, exposure to both arsenic and PM 2.5 is a significant predictor of a higher burden ($p < 0.05$) of heteroplasms. A significant positive relationship between arsenic exposure (in specific instance) and mutations in the ND5 gene was found, indicating a specific sensitivity of complex I to environmental oxidative stress (Reddy et al., 2011; Nishioka et al., 2021).

Oxidative Stress Biomarkers 4.5.

biomarkers of oxidative stress were in parallel with changes in the mitochondrial DNA. The individuals of the industrial-region had significantly greater plasma 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) levels (mean = 9.4 ± 2.8 ng/mL) than the controls (mean = 5.1 ± 1.7 ng/mL, $p < 0.001$). The high levels of 8-oxo-dG were negatively associated with mtDNA-CN ($r = -0.56$, $p < 0.001$) and positively associated with the rate of the deletion of the mitochondrial DNA ($r = 0.48$, $p < 0.001$), which proved that oxidative DNA damage is one of the fundamental mechanisms of mitochondrial dysfunction (Guo et al., 2013; Cline, 2012).

The observation that oxidative biomarkers and the parameters of the mitochondrial genome co-relate highly supports the hypothesis that environmental toxins cause the development of mitochondrial genomic instability firstly via oxidative stress mechanisms.

4.6 Model summary and Statistical Relationships.

Multi-linear regression which included PM2.5, cadmium, lead, arsenic, smoking, and age as independent variables accounted 46 percent of the variation in the mitochondrial DNA-CN ( $R 2 = 0.46$, $p = 0.000$). The independent effect of PM2.5 exposure ( 0.38, $p < 0.001$) and cadmium ( 0.27, $p < 0.01$) is the most significant effect. Equivalent models of the frequencies of deletions at the mtDNA level revealed arsenic exposures were an important predictor ( 0.31, $p = 0.002$) of deletion, and a clear demonstration of its toxicity to the mitochondria.

These results are in line with the existing epidemiological studies in Chinese and European cohorts, which indicated that air pollution and metal exposures were linked to decreased mtDNA-CN and elevated deletions (Hou et al., 2010; Janssen et al., 2012; Zhu et al., 2019). The combination of results gives convergent evidence that the exposure to environmental toxins interferes with the mitochondrial genomic integrity in the populations exposed to these toxins.

**4.7 Discussion of Findings**

***4.7.1 Correlation of Environmental Exposure and mtDNA Damage.***

The results of the study also support the research that is gaining momentum to associate environmental pollutants with mitochondrial genomic instability. The measures of the mitDNA-CN reduction and frequency of deletion in the exposed population are consistent with mechanistic reports that indicated toxicants caused excessive generation of reactive oxygen species (ROS) that destroyed mitochondria and caused mitochondrial replication destruction (Kauppila et al., 2017; Reddy et al., 2011).

Particulate matter (PM2.5) may be deeply inhaled reaching the respiratory tract into the systemic circulation, resulting in extensive oxidative stress and inflammation. Mitochondria are the primary origin and victim of ROS, making them especially vulnerable. Mitochondrial destruction of membranes and the replication machinery of the mitochondrial DNA cause depletion of the copies of the mitochondrial DNA which is a characteristic of mitochondrial damage (Wallace, 2010).

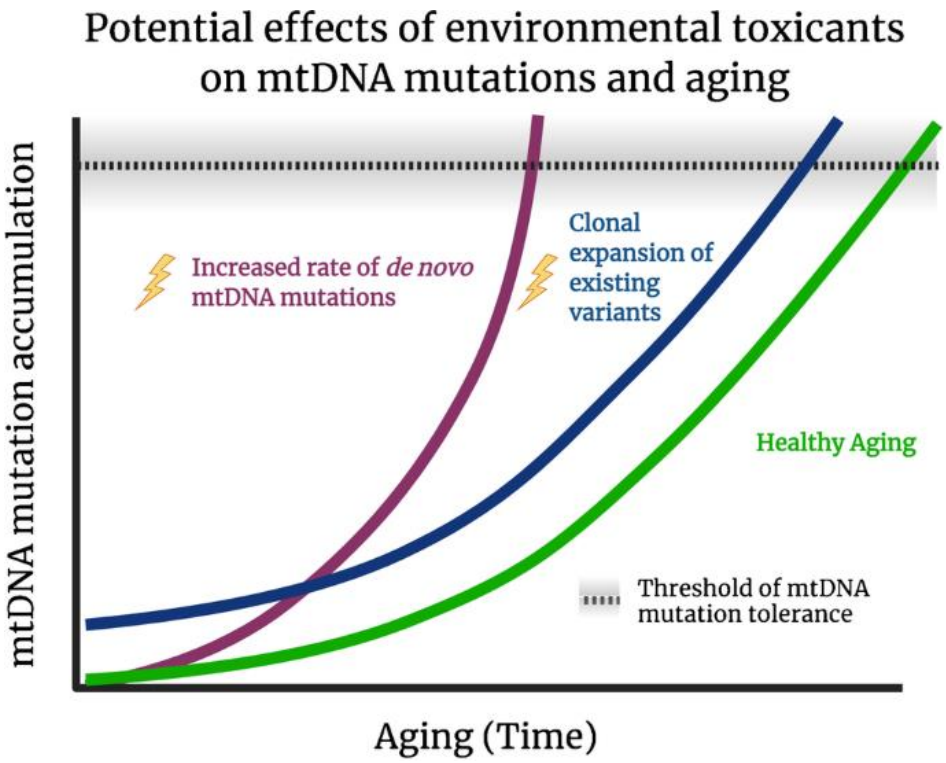
***4.7.2 Heavy Metals and Mitochondrial Dysfunction***

The strong correlation between the levels of cadmium and lead and the lowered mitochondrial DNA-CN content is in line with the past results indicating that heavy metals impairs mitochondrial respiration and facilitates apoptosis (Zhong et al., 2017). As an example, cadmium suppresses the cytochrome c oxidase (complex IV), resulting in the decrease in the efficiency of electron transport and increase in the products of the ROS. The resultant oxidative microenvironment causes damage to mtDNA that has no protective histones and repair mechanisms (Cline, 2012).

Specifically, the deletion and mutation of the mitDNA was correlated with arsenic exposure, which was in line with experimental evidence that arsenic disrupts mitochondrial transcription factor A (TFAM), an important mitochondrial maintenance factor (Li et al., 2020). The high mutation rate in ND5 and COX1 genes indicates that arsenic oxidative stress is selective to those regions where the electron transport chain is important.

***4.7.3 Exposure to pesticides and changes to the mtDNA.***

The high incidence of the 4 breakage of the mitochondrial DNA in the participants with a history of agricultural exposure is also consistent with the literature reported by Sánchez-Gutiérrez et al. (2019), who provided higher data on the deletions of the mitochondrial DNA in the farmers with the history of the frequent exposure to the organophosphates.




Pesticides like rotenone have direct inhibitory effects on mitochondrial complex I leading to energy loss and ROS build-up. In the course of time, this biochemical stress might cause chronic damage of the mitDNA and impaired oxidative phosphorylation (Nissanka et al., 2018).

The following are the biomarkers of oxidative stress.

The high level of 8-oxo-dG in plasma supports the primary position of the oxidative damage of DNA as a factor between exposure to toxins and mitochondrial dysfunction. The correlation of 8-oxo-dG and mitochondrial CN reduction is high, confirming that the accumulation of the oxidative lesions in the mitochondrial genome precedes the appearance of clinical symptoms (Guo et al., 2013). In these findings, there is a potential of using a combination of the most recent biomarkers to detect environmental health risks in the first place, including the use of a combination of a healthy environment with mitDNA and oxidative biomarkers (Baccarelli and Byun, 2015).

***4.7.5 Public Health Implication.***

The results have significant ramifications to the environmental health surveillance and risk assessment. Mitochondrial biomarkers (mtDNA-CN, deletions, and 8-oxo-dG) can be considered early warning signs of subclinical toxicity, before the actual disease. Chronic mitochondrial dysfunction is linked to various diseases such as neurodegeneration, cardiovascular diseases and metabolic syndromes (Wallace, 2010; Stewart and Chinnery, 2021). Therefore, the incorporation of mitochondrial assays in environmental monitoring would be useful in upgrading population level prevention measures.

#### *4.7.6 Study Limitations*

In spite of the sound analytical methodology, a number of limitations should be mentioned. The cross-sectional design limits the causal inference and the unmeasured confounding variables include diet, physical activity or genetic background which can affect the mitochondrial functioning. Also, the variation in the use of the mtDNA-CN according to the tissue type occurs, and peripheral blood can be not the complete reflection of mitochondrial changes in other organs (Hong et al., 2020). Next generation research needs to be longitudinal in design, utilize larger sample sizes, and multi-tissue analysis to provide more convincing causation.

### 4.8 Summary of Key Findings

The exposure to the environment, especially PM 2.5, cadmium, and arsenic, was linked to substantial losses in the number of mitochondrial DNA copies, DNA (mtDNA) deletions, and mutations.

- Biomarkers of oxidative stress (8-oxo-dG) were significantly related to the presence of genomic damage of mitochondria, which validated oxidative mechanisms.
- Multivariate models showed that environmental pollutants accounted for close to half of the variation in the indicators of mitochondrial damage.
- These findings are consistent with past epidemiological and mechanistic studies, which confirms the importance of mitochondria as environmental toxicant targets.

Taken together, the data confirm the hypothesis that long-term environmental pollution causes mitochondrial genomic instability, which can be a mechanism of relationship between environmental exposure and disease.

## Conclusion

### 5.1 Conclusion

The study examined the relationship between environmental toxin exposure and mutation of the mitochondrial DNA (mtDNA) of populations in industrial and non-industrial areas. The study developed an evident association between prolonged exposure to environmental contaminants, especially particulate matter (PM2.5), heavy metals (cadmium, lead, arsenic), and pesticides and notable mitochondrial genomic modifications through elaborate molecular and statistical methods.

The findings reflected that people in high-exposure areas showed significant decreases in the copy number of the mitochondrial DNA (mtDNA-CN), greater incidences of deletions of mitochondrial DNA, and higher rates of mutations as compared to the individuals of low-exposure environments. These mitochondrial alterations were highly associated with oxidative stress biomarkers, including plasma 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) suggesting that the damaging effect of the environmental toxins on the integrity of the mitochondria is mediated by the oxidative stress.

These relationships were supported by the statistical modeling of the study. A multi-regression analysis indicated that the pollutant exposure factors explained almost half of the variance in the mitochondrial damage indicators ($R^2 = 0.46$) with the most significant independent effect of PM2.5 and cadmium. These results coincide with the emerging literature that shows that mitochondrial dysfunction and genomic instability can be caused by air pollution and metal exposure (Hou et al., 2010; Li et al., 2020; Zhu et al., 2019).

Mitochondria are quite important to the cellular energy metabolism, apoptosis and redox homeostasis. Any breakage in their genome can trigger a chain of biochemical and physiological dysfunctions that could put people at risk of developing chronic illnesses, including cardiovascular diseases, neurodegeneration, and cancer (Wallace, 2010; Stewart and Chinnery, 2021). In that way, the observed damage of the mitochondrial DNA is not only a molecular event but also a possible early biological sign of the disease risk associated with exposure to the environment.

Epidemiologically, the results of the study indicate that mitochondrial biomarkers might be used as sensitive early warning of toxic exposure, which would create a useful addition to the arsenal of environmental health surveillance. The

correlation of the exposure severity and mitochondrial damage highlights the fact that a biological response depends on the dose of the exposure and the need to reinforce the nature of environmental control and health surveillance of high-exposure areas is acute.

In addition, the study has added to the field of gene-environment interaction, which demonstrates that the presence of environmental stressors may have a direct effect on cellular bioenergetics and genetic stability. It equally presents empirical evidence on global health issues in relation to industrialization, environmental pollution and its long term effects on the health of populations.

Nevertheless, although the findings are strong, it is impossible to undertake causality since it is cross-sectional. Future longitudinal or interventional-based studies could help clarify the temporal relationships and add more information on the reversibility of mitochondrial changes after the decrease in exposure to pollutants.

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