Assessment of urinary oxidative stress biomarkers associated with fine particulate matter (PM2.5) exposure in Chiang Mai, Thailand (#106470)

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Background: Exposure to fine particulate matter (PM2.5) is known to increase oxidative stress, impacting health adversely. This study examines the relationship between PM2.5 exposure and oxidative stress biomarkers in Chiang Mai, Thailand.

Methods: A pilot prospective observational study was conducted in Samoeng District, Chiang Mai, including 25 healthy participants (age 25-60 years). Urine samples were collected during high (Feb-April 2023) and low (May-July 2023) PM2.5 seasons. PM2.5 concentrations were monitored daily from the NTAQHI website. Biomarkers analyzed included 1-hydroxypyrene (1-OHP) using HPLC, malondialdehyde (MDA) via Spectrophotometry, and 8-epi-prostaglandin F2α (8-epi-PGF2α) with ELISA. Statistical analysis was performed using IBM SPSS Statistics 22.0.

Results: Significant increases in urinary 1-OHP, MDA, and 8-epi-PGF2α were observed during the high PM2.5 season compared to the low season. The mean concentration of PM2.5 was 67 μg/m³ during high pollution and 7 μg/m³ during low pollution. Elevated levels of these biomarkers indicate increased oxidative stress associated with higher PM2.5 exposure.

Conclusions: This study highlights a significant association between elevated PM2.5 levels and increased oxidative stress biomarkers in Chiang Mai, Thailand. The findings suggest that exposure to higher concentrations of PM2.5 contributes to oxidative stress, potentially leading to adverse health outcomes.

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Abstract

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Introduction

Air pollution, resulting from both human activities and natural sources such as vehicle and industrial emissions, agricultural residue burning, biomass burning, and forest fires, has significant transboundary effects, especially in regions like Southeast Asia (Thongtip, Srivichai et al. 2022). Globally, ambient air pollution is a critical issue, contributing to climate change and public health crises (Altuwayjiri, Taghvaee et al. 2021), and accounts for an estimated 7 million premature deaths every year [WHO,2021]. In developing countries, rapid urbanization and

industrialization have exacerbated this problem, leading to increased exposure to pollutants like PM2.5, which poses severe health risks (Sukkhum, Lim et al. 2022).

PM2.5, fine particulate matter or particulate matter with a diameter of less than 2.5 micrometers, is particularly concerning due to its ability to penetrate deep into the respiratory system and enter the bloodstream (Amnuaylojaroen and Parasin 2023). Its small size and large surface area enable PM2.5 to carry various toxic substances, including polycyclic aromatic hydrocarbons (PAHs), which are known to induce oxidative stress and inflammation. Epidemiological and toxicological studies have linked PM2.5 exposure to a range of health issues, including cardiovascular diseases, respiratory problems, and various forms of cancer (Bhatnagar 2022). Additionally, oxidative stress and DNA damage are critical mechanisms through which PM2.5 exerts its harmful effects, facilitated by reactive oxygen species (ROS) production (Liu, Jiang et al. 2024).

Figure 1 Mechanism of oxidative stress induced by PM2.5 exposure, showing the imbalance between ROS and antioxidants leading to oxidative damage.

The mechanism of oxidative stress illustrated in Figure 1 induced by PM2.5 and PAHs is a critical pathway in understanding the health impacts of air pollution. As illustrated in Figure 1, the small size of PM2.5 allows it to penetrate deep into the respiratory system and enter the bloodstream, where it can transport toxic substances such as PAHs. These substances can generate ROS through processes like the redox cycle, leading to oxidative stress, which in turn induces DNA and lipid damage. This cascade of events is linked to various adverse health outcomes, including inflammation, cardiovascular diseases, and cancer (Li, Xia et al. 2008, Møller and Loft 2010). Understanding this mechanism is vital for identifying potential biomarkers, such as 1-hydroxypyrene (1-OHP), malondialdehyde (MDA), and 8-isoprostaglandin F2 alpha (8-iso-PGF2α), which can be used to monitor exposure and assess the biological impact of PM2.5 on human health.

In Thailand, transportation, industries, and biomass burning are the main sources of emissions (Sirithian and Thanatrakolsri 2022). PM2.5, PM10, ozone (O_3), and volatile organic compounds (VOCs) are examples of air pollutants exceeding national ambient air quality

standards. Although air quality improved in 2020, levels remained above standard for over 70 days, primarily in northern provinces. Residents suffer respiratory issues due to haze pollution during the dry season due to open burning and natural forest fires. Northern Thailand's mountainous cities, surrounded by farming, generate a lot of air pollution (Amnuaylojaroen, Parasin et al. 2022).

PAHs have been shown to create ROS through the redox cycle, which can induce oxidative modification of DNA and lipids in vivo (Fujitani, Furuyama et al. 2023). PAHs are produced by biogenesis and human activities, such as incomplete combustion of fossil fuels, industrial processes, and biomass burning (Tala, Kraisitnitikul et al. 2023). Urinary 1-OHP, a metabolite of pyrene, is a useful biomarker for PAH exposure (Kho, Lee et al. 2015). In several studies, 1-OHP levels were utilized to biologically monitor PAH uptake from occupational exposures (Brucker, Moro et al. 2013). The study provides an overview of 1-OHP's association with PAH exposure.

Biomarkers like MDA and 8-iso-PGF2α have been widely used in studies assessing oxidative stress related to air pollution exposure (Zhao, Gong et al. 2018). MDA is a well-known biomarker for oxidative stress, and its elevated levels have been linked to exposure to pollutants like PM2.5 and O_3. Studies have demonstrated increased levels of MDA in the ear and brain following exposure to wood smoke, as well as in asthmatic children exposed to PM2.5 and O_3 (Toto, Wild et al. 2022). Urinary MDA measurements have also been utilized to evaluate air pollution exposure, primarily focusing on subjects with respiratory diseases, children, or the elderly. Despite its potential, the application of MDA as a biomarker in epidemiological studies concerning air pollution remains relatively limited.

On the other hand, 8-iso-PGF2α, part of the F2-isoprostane family, is a nonenzymatic product of arachidonic acid peroxidation and is recognized as a sensitive biomarker for oxidative status (Il'yasova, Scarbrough et al. 2012). Elevated urinary levels of specific isomers, such as iPF2α-III and iPF2α-VI, have been found in individuals with various health conditions, including cardiovascular disease, Alzheimer's disease, type 2 diabetes, Down syndrome, lung disorders, and heavy smokers (Zhang, Il'yasova et al. 2010). The metabolite 2,3-dinor-8-iso-prostaglandin F2α is particularly useful as a biomarker for the formation of 8-iso-PGF2α and lipid peroxidation

in vivo, providing insights into the oxidative damage occurring within the body due to environmental and other stressors.

Chiang Mai, particularly the Samoeng district, experiences significant air pollution issues, primarily during the dry season due to agricultural residue burning and forest fires (Jainonthee, Wang et al. 2022). The region's topography, characterized by mountains and valleys, exacerbates air quality problems, trapping pollutants and leading to extended periods of poor air quality (Supasri, Gheewala et al. 2023). The local population frequently experiences health issues such as respiratory diseases and ocular surface diseases, associated with high levels of PM2.5 and other pollutants. Despite these challenges, there is limited data on the health impacts of air pollution in this region, particularly concerning oxidative stress biomarkers.

Given the significant impact of PM2.5 on public health and the limited data available on oxidative stress biomarkers associated with air pollution in Chiang Mai, this study aims to investigate the relationship between PM2.5 exposure and oxidative stress biomarkers in the region. The primary objectives are to assess urinary levels of 1-OHP, MDA, and 8-iso-PGF2α, evaluate their association with PM2.5 exposure, and explore the implications for public health in the Samoeng district. This research seeks to provide valuable insights into the biological effects of air pollution, contributing to developing effective strategies for mitigating its adverse health impacts in northern Thailand.

Materials and Methods

Study Area

The focus area was chosen to be Chiang Mai province (Jarernwong, Gheewala et al. 2023). With a total area of 20,107 km^2, it is the largest province in the north of Thailand. The geographical location of the Chiang Mai province is shown in Figure 2. It has 25 districts, with a total population of roughly 1.78 million based on the Department of Provincial Administration of Thailand in 2020.

A unique area of Chiang Mai province, Samoeng District, was selected for sample collection because of its unique environment, including higher levels of particulate matter PM2.5 during

the burning season, as well as its rural and agricultural setting, making it a perfect location to study health impacts of pollution (Paesrivarotai and Tanaksaranond 2021).

Figure 2 Map showing the study area in Chiang Mai, Thailand.

Study Population

This is a pilot, prospective observational study including 25 healthy participants from the Samoeng district of Chiang Mai province in Thailand. Participants were selected based on predefined inclusion and exclusion criteria to ensure a homogeneous sample of healthy individuals aged between 25-60 years. Exclusion criteria included any underlying diseases, recent operations, certain chronic conditions, pregnancy, drug abuse, psychological disorders, and infections.

Data Collection

Data collected from participants includes demographics (age, gender, smoking, alcohol drinking, underlying diseases, marital status, education, occupation, family income and financial support), physical examination (height, weight, BMI, waist circumference, hip circumference, diastolic BP, systolic BP, and heart rate). Urine samples were examined for biomarkers (1-OHP, MDA, and 8-iso-PGF2α) detection.

Urine samples were collected during high (Feb-April 2023), and low (May-July 2023) PM2.5 seasons, which were provided by Asst. Prof. Kanokwan Kulprachakarn, Ph.D., from the research project entitled “Health Risk Assessment and Association between Metabolic and Hormonal Derangements in People Exposed to In-house or Ambient PM2.5-Bond Chemicals”. The samples were stored at -20 °C until further analysis.

The concentrations of PM2.5 was measured and recorded daily, Air pollution data on a daily basis were obtained from the Northern Thailand Air Quality Index (NTAQI)

(https://www2.ntaqhi.info/). In order to conduct this study, the authors calculated the monthly average.

Biomarkers Analysis

Creatinine (Cr)

The amount of Cr in the samples was measured using the spectrophotometric Jaffé method, which relies on the reaction of Cr with picric acid in an alkaline pH solution (Campos, Guzmán et al. 2011). For this purpose a creatinine assay kit (Colorimetric) (ab204537) was used which is a complete kit for the quantitative determination of creatinine in urine.

1-Hydroxypyrene (1-OHP)

The stored urine samples were used for 1-OHP analysis with modifications by the method of K. Sutan, W. Naksen, and T. Prapamontol (Sutan, Naksen et al. 2017) . Specifically, 2.5 mL of urine was adjusted to pH 5.0 using 1M HCl and then transferred into a 50 mL screw cap test tube containing 2.5 mL of 0.1M acetate buffer and 6.25 µL of β-glucuronidase from Helix pomatia. The mixture was vortexed for 10 seconds and incubated at 37°C for 2 hours. After incubation, the samples were processed using a Vertipak C18 3 mL solid-phase extraction (SPE) cartridge. The cartridge was pre-conditioned by rinsing it with 1 mL of methanol (three times), followed by 1 mL of water (three times). The samples were then loaded onto the cartridge and washed with 2.5 mL of water, followed by 2.5 mL of 20% methanol. Samples were loaded onto the cartridge and washed with 2.5 mL of water and 2.5 mL of 20% methanol. The cartridge was allowed to stand for 10 minutes before elution with 2.5 mL of pure methanol. The eluted solution was filtered through a 0.2 µm PTFE syringe filter before being evaporated to dryness under a stream of nitrogen gas, then reconstituted in 100 µL of methanol. For HPLC analysis, a 20-µL injection was performed using an HPLC system from Agilent 1260 Infinity. The mobile phase consisted of 45% water (Line A) and 55% acetonitrile (Line B) at a flow rate of 0.80 mL/min. Chromatographic separation was achieved using an InfinityLab Poroshell 120 EC-C18 (4.6 mm x 150 mm, 5 µm) maintained at 25 °C. The detection was carried out using a fluorescence detector with an excitation wavelength of 242 nm and an emission wavelength of 388 nm, and

the total run time was 20 minutes. This technique was modified to efficiently process smaller sample volumes while maintaining accurate and reliable quantification of 1-OHP.

The standard curve was created using the 1-OHP standards' concentration range of 0.00125 to 2.50 ng/mL. The calibration equation was obtained by linear regression, with the signal response (peak area) plotted against known concentrations ($y = 1.74x + 2.24799\times10-2$, $R2 = 0.983$). This calibration equation served as the foundation for the sample concentration calculations. Specifically, the limits of detection (LOD) and limits of quantitation (LOQ) were 0.2634 and 0.7984 ng/ml, respectively.

Thio barbituric acid-reacting substances (TBARS)

TBARS were measured by the method of C. Campos et al. (Campos, Guzmán et al. 2011). In summary, 140 µL of urine was mixed in a vortex with 33 µL of 0.01% BHT (in absolute ethanol), 1 mL of 1% phosphoric acid, and 300 µL of 42 mmol/L TBA (dissolved in water and heated). Following a 45-minute incubation period in boiling water, 1.4 mL of 1-butanol was added to each tube and the tubes were allowed to cool on ice. Using a UVmini-1240 Shimadzu spectrophotometer (Shimadzu, Tokyo, Japan), the absorbance of the supernatant was measured at 535 nm after a 15-minute centrifugation (2000 × g). To create the standard absorption curve, MDA was dissolved in 20 mmol/L of phosphate buffer (pH 7.0).

8-Epi-Prostaglandin F2 Alpha (8-epi-PGF2α)

Urinary 8-iso-PGF2α was determined by the commercial ELISA kit (ElabScience) according to the manufacturer’s instruction. The ELISA kit uses the Competitive-ELISA principle, using a pre-coated micro plate with 8-epi-PGF2α. The enzyme competes with a fixed amount on the solid phase supporter for specific sites. Excess conjugate and unbound sample are washed away, Avidin-Horseradish Peroxidase conjugate is added, and TMB substrate solution is added. Optical density (OD) is measured.

Ethical Considerations

The study protocol was approved by Human Experimentation Committee Research Institute for Health Sciences (RIHES), Chiang Mai University, Chiang Mai, Thailand on 19 January 2023 [Project No.03/2023]. Written informed consent was obtained from all subjects involved in the study.

Statistical Analysis

The data are shown as mean ± standard deviation (SD). The Shapiro–Wilk test was used to determine whether the data had a normal distribution. The Mann-Whitney U-test was used for nonparametric data, and the Student's t-test was utilized for parametric variables. $P < 0.05$ was designated as the threshold for statistical significance. IBM SPSS Statistics 22.0 was used to process the data.

Results

Baseline Characteristics of Participants

This study assessed 25 healthy participants from the Samoeng district in Chiang Mai province, Thailand. The participants had a mean age of 48.1 years (SD = 14.6), with a gender distribution of 60% female and 40% male. Most participants were smokers (84%), and 24% reported alcohol consumption. Educational backgrounds varied, with 44% having less than a high school education, 44% holding college or university degrees, and 12% having completed high school. The occupational breakdown showed that 52% of participants worked in agriculture, 12% in service, and 36% in other sectors. Table 1 shows the baseline characteristics of the participants in terms of mean and standard deviation for ~~continues~~ variables and frequency and percentage for categorical variables.

Table 1 Baseline characteristics of the participants (N = 25).

240 **PM2.5 Concentration During High and Low Pollution Seasons**

241 The daily PM2.5 concentration data were obtained from the NTAQHI website, managed by
242 RIHES CMU, for the periods of March-April and May-July 2023. The mean concentrations of
243 PM2.5 during both high and low pollution seasons are presented in Figure 3. During the high
244 pollution season (March-April 2023), PM2.5 levels were significantly elevated, with the highest
245 concentration observed in April (88.3 μg/m³), followed by March (67.3 μg/m³). In contrast, the
246 low pollution season (May-July 2023) exhibited substantially lower PM2.5 concentrations, with
247 mean levels of 15.8 μg/m³ in May and 4.1 μg/m³ in July. The variation between these months
248 highlights the distinct seasonal air quality differences in the Samoeng District of Chiang Mai.

249 This variation in PM2.5 levels between the two seasons was critical for analyzing the seasonal
250 impact of air pollution on urinary oxidative stress biomarkers in participants. The average PM2.5
251 concentration for the high pollution season was calculated at 67 μg/m³, while the low pollution
252 season averaged 7 μg/m³. These differences provided a strong basis for assessing the correlation
253 between PM2.5 exposure and biomarker levels, particularly for oxidative stress markers such as
254 1-OHP, MDA, and 8-epi-PGF2α.

255

256 **Figure 3 Mean concentrations of PM2.5 (μg/m³) recorded during high (March-April 2023)**
257 **and low (May-July 2023) pollution seasons.** The error bars represent the standard error of the
258 mean (SEM).

259

260 **Urinary Biomarkers and PM2.5 Concentration by Season**

261 In this study, the authors observed significant differences in the urinary concentrations of
262 oxidative stress biomarkers between high and low PM2.5 seasons, which are depicted in Figure
263 4. The graph presents the median concentrations of 8-epi-PGF2α, 1-OHP, and MDA across the
264 two seasons, highlighting the impact of seasonal PM2.5 exposure on these biomarkers.

265

Figure 4 Comparison of urinary concentrations of oxidative stress biomarkers, including A) 8-epi-PGF2α, B) Malondialdehyde (MDA), and C) 1-hydroxypyrene (1-OHP) between high PM2.5 and low PM2.5 seasons. Data are presented as median values with interquartile ranges (Q1, Q3) for each biomarker. The Wilcoxon signed-rank test was used to assess the significance of differences between seasons, with all *p-values < 0.05 indicating statistically significant increases in biomarker levels during the high PM2.5 season.

The concentration of 8-epi-PGF2α, measured in pg/mg creatinine, was significantly higher during the high PM2.5 season (median = 139.43 pg/mg, Q1 = 80.63, Q3 = 187.54) compared to the low PM2.5 season (median = 54.22 pg/mg, Q1 = 26.74, Q3 = 122.76), with a p-value of 0.016. This indicates an elevated oxidative stress level during periods of higher air pollution.

The concentration of malondialdehyde (MDA), measured in μM/mg creatinine, was also significantly higher during the high PM2.5 season (median = 3.15 μM/mg, Q1 = 2.83, Q3 = 4.11) compared to the low PM2.5 season (median = 2.45 μM/mg, Q1 = 1.96, Q3 = 3.05), with a p-value of 0.006. The elevated MDA levels during high PM2.5 exposure further support the hypothesis that increased air pollution contributes to oxidative stress.

Similarly, the urinary concentration of 1-hydroxypyrene (1-OHP), expressed in mg/g creatinine, showed a significant increase during the high PM2.5 season (median = 0.09 mg/g, Q1 = 0.06, Q3 = 0.17) compared to the low PM2.5 season (median = 0.04 mg/g, Q1 = 0.02, Q3 = 0.11), with a p-value of 0.001. The increased levels of 1-OHP suggest higher internal exposure to polycyclic aromatic hydrocarbons (PAHs) during periods of elevated PM2.5 levels.

The Wilcoxon signed-rank test was applied to assess these differences, and all p-values were found to be significant, indicating a robust association between increased PM2.5 exposure and elevated urinary biomarkers of oxidative stress. These findings underscore the heightened oxidative stress during periods of high air pollution, particularly in the Samoeng District, where residents, predominantly farmers, are more susceptible to PM2.5 exposure due to agricultural practices like stubble burning.

This analysis provides clear evidence of the health risks associated with seasonal variations in PM2.5 levels, reinforcing the need for targeted interventions to reduce exposure, particularly in

vulnerable populations. The significant changes in these biomarkers reflect the biological impact of air pollution and suggest potential pathways through which PM2.5 exposure may lead to adverse health outcomes.

Biomarkers in Relation to PM2.5 Exposure, Age, Gender, and Smoking Status

To further investigate the relationship between PM2.5 exposure and oxidative stress biomarkers, we employed a Generalized Estimating Equations (GEE) model, adjusting for potential confounders such as age, gender, and smoking status as shown in Table 2. The analysis revealed significant associations for some biomarkers, underscoring the potential health impacts of air pollution in the study population.

1-Hydroxypyrene (1-OHP)

The regression analysis showed a positive association between PM2.5 levels and 1-OHP, with an estimated coefficient (Exp(b)) of 1.014 (95% CI: 1.01, 1.02, $p < 0.01$). This suggests that for each unit increase in PM2.5 concentration, the level of 1-OHP increases by approximately 1.4%. This finding highlights the strong influence of PM2.5 on PAH metabolism, potentially leading to elevated oxidative stress. Other variables, such as age (Exp(b) = 0.992, $p = 0.422$), gender (Exp(b) = 1.03, $p = 0.933$), and smoking status (Exp(b) = 0.77, $p = 0.543$), did not show significant associations with 1-OHP levels, indicating that PM2.5 is a more critical factor in influencing this biomarker.

Malondialdehyde (MDA)

The association between PM2.5 and MDA was not statistically significant, with a coefficient of 0.998 (95% CI: 0.997, 1.00, $p = 0.051$). However, the p-value approaches significance, suggesting a potential negative relationship that warrants further investigation. Interestingly, age was significantly associated with MDA levels (Exp(b) = 1.01, $p = 0.007$), indicating that older participants may have higher oxidative stress levels, independent of PM2.5 exposure. Gender also showed a significant association (Exp(b) = 1.15, $p = 0.041$), with females exhibiting higher MDA levels compared to males. Smoking status, however, did not significantly influence MDA levels (Exp(b) = 1.05, $p = 0.583$).

8-iso-Prostaglandin F2α (8-iso-PGF2α)

A significant positive association was found between PM2.5 and 8-iso-PGF2α levels (Exp(b) = 1.008, 95% CI: 1.00, 1.02, p = 0.045). This suggests that increased PM2.5 exposure is linked to elevated levels of 8-iso-PGF2α, a biomarker of lipid peroxidation, reinforcing the role of PM2.5 in promoting oxidative stress. Age and gender did not show significant associations with 8-iso-PGF2α levels (Exp(b) = 1.013, p = 0.196 and Exp(b) = 1.377, p = 0.344, respectively). However, smoking status showed a marginal association (Exp(b) = 2.22, p = 0.081), indicating a trend towards higher 8-iso-PGF2α levels among smokers.

Overall, these findings demonstrate that PM2.5 exposure is a significant determinant of certain oxidative stress biomarkers, particularly 1-OHP and 8-iso-PGF2α. The absence of significant associations with MDA may suggest different pathways or sensitivities among the biomarkers. This analysis underscores the complex interactions between environmental pollutants and biological responses, highlighting the importance of targeted interventions to reduce PM2.5 exposure and its associated health risks.

Table 2 Multivariate regression analysis shows significant associations between PM2.5 exposure and elevated levels of urinary oxidative stress biomarkers, even after adjusting for age, gender, and smoking status.

Note: 1-OHP: 1-hydroxypyrene; MDA: Malondialdehyde; 8-iso-PGF2α: 8-iso-prostaglandin-F2α.

*P-values < 0.05 indicating statistically significant association.

Discussion

This pilot study provides novel insights into the relationship between PM2.5 exposure and oxidative stress biomarkers in Chiang Mai, Thailand. Our findings demonstrate that elevated PM2.5 levels are significantly associated with increased concentrations of 1-OHP, MDA, and 8-iso-PGF2α, indicating a pronounced oxidative stress response. The observed increase in 1-OHP and 8-iso-PGF2α levels during periods of high PM2.5 exposure corroborates previous studies linking air pollution to oxidative stress. PM2.5 carries various toxic substances, including PAHs,

which are metabolized into 1-OHP, a reliable biomarker for PAH exposure (Luo, Stepanov et al. 2019, Liu, Liu et al. 2023). Similarly, the significant elevation in 8-iso-PGF2α, a marker of lipid peroxidation, aligns with research highlighting the role of air pollution in inducing oxidative damage and inflammation (Glencross, Ho et al. 2020, Leni, Künzi et al. 2020).

The correlation between PM2.5 levels and MDA, though less pronounced than for 1-OHP and 8-iso-PGF2α, still underscores the oxidative stress induced by particulate matter exposure. MDA, a byproduct of lipid peroxidation, has been widely used as a biomarker for oxidative stress in various studies, further validating our findings(Cui, Gong et al. 2018, Zhang, Liu et al. 2023). The impact of PM2.5 on oxidative stress biomarkers, as evidenced by our study, suggests potential health risks, including cardiovascular and respiratory diseases, which have been extensively documented in the literature(Brook, Rajagopalan et al. 2010, Schraufnagel, Balmes et al. 2019).

Our results also reveal a significant association between age and MDA levels, with older individuals exhibiting higher levels of oxidative stress. This finding is consistent with previous studies showing age-related susceptibility to oxidative damage due to the cumulative effects of environmental exposures over time (Weary 2023) . Gender differences were observed, with females showing higher MDA levels, a finding that resonates with research suggesting that hormonal differences, particularly the presence of estrogen, may influence oxidative stress responses(Viña, Borrás et al. 2005, Berry 2022).

Interestingly, no significant association was found between smoking status and the oxidative stress biomarkers studied, although a trend was noted with 8-iso-PGF2α. This may be attributed to the small sample size and the possibility that oxidative stress induced by PM2.5 exposure could overshadow the effects of smoking. The literature reports mixed results, with some studies showing a synergistic effect of smoking and air pollution on oxidative stress, while others have not (Peretz, Kaufman et al. 2008, Makra, Puskás et al. 2015).

Overall, our findings are consistent with a growing body of evidence linking air pollution, particularly PM2.5, to oxidative stress and the development of non-communicable diseases (NCDs). Oxidative stress is a key mechanism through which PM2.5 exposure contributes to the pathogenesis of cardiovascular and respiratory diseases, as well as cancer (Wang, Ma et al. 2019). The specific biomarkers analyzed in our study—1-OHP, MDA, and 8-iso-PGF2α—are

widely recognized as indicators of oxidative stress, providing a robust framework for assessing the biological impact of air pollution (Zhao, Xu et al. 2023).

The findings of this study have important public health implications, particularly in regions like Chiang Mai, where seasonal air pollution is a significant concern. The association between PM2.5 exposure and oxidative stress biomarkers suggests that residents in highly polluted areas are at an increased risk of developing oxidative stress-related diseases. This underscores the need for targeted interventions to reduce air pollution exposure, particularly during the burning season. Additionally, the use of oxidative stress biomarkers in epidemiological studies could provide valuable insights into the long-term health effects of air pollution and inform public health policies aimed at mitigating these risks.

The strengths of this study lie in its focused, area-specific approach, targeting the Samoeng District, a region previously unexplored in this context. This district, primarily inhabited by farmers, presents a unique population with heightened exposure to PM2.5, particularly due to stubble burning, making the findings highly relevant to local environmental health concerns. The study's design, with repeated measurements during different PM2.5 seasons, allows for the observation of seasonal variations in oxidative stress biomarkers, providing a comprehensive view of the impact of air pollution on health. Additionally, the use of multiple biomarkers—8-epi-PGF2α, MDA, and 1-OHP—strengthens the evidence of oxidative stress due to PM2.5 exposure, and the significant correlations observed add to the robustness of the study's findings.

However, this study has some limitations. One of the primary limitations is the small sample size, which limits the generalizability of the findings. As a pilot study, the results should be interpreted with caution, and larger studies are needed to validate these findings. Furthermore, the study did not account for other potential confounding factors, such as diet, physical activity, and socioeconomic status, which could influence oxidative stress levels. The cross-sectional design of the study also limits the ability to draw causal inferences between PM2.5 exposure and oxidative stress biomarkers.

Future research should focus on expanding the sample size and incorporating longitudinal data to better understand the temporal relationship between PM2.5 exposure and oxidative stress. Additionally, exploring the role of other potential confounders, such as genetic susceptibility and lifestyle factors, could provide a more comprehensive understanding of the factors influencing

oxidative stress. Investigating the combined effects of air pollution and other environmental exposures, such as indoor air pollution and chemical contaminants, could also shed light on the cumulative impact of multiple stressors on oxidative stress and health outcomes.

Conclusion

In conclusion, this study highlights the significant impact of PM2.5 exposure on oxidative stress biomarkers, with higher levels observed during the high PM2.5 season in the Samoeng District. The strong associations found between PM2.5 and biomarkers such as 8-epi-PGF2α, 1-OHP, and MDA reinforce the health risks posed by air pollution, particularly in rural areas prone to stubble burning. These findings underscore the urgent need for targeted strategies to mitigate air pollution and protect public health in vulnerable communities.

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References

Altuwayjiri, A., S. Taghvaee, A. Mousavi, M. H. Sowlat, M. S. Hassanvand, H. Kashani, S. Faridi, M. Yunesian, K. Naddafi and C. Sioutas (2021). "Association of systemic inflammation and coagulation biomarkers with source-specific PM2. 5 mass concentrations among young and elderly subjects in central Tehran." Journal of the Air & Waste Management Association **71**(2): 191-208.

Amnuaylojaroen, T. and N. Parasin (2023). "Future health risk assessment of exposure to PM2. 5 in different age groups of children in Northern Thailand." Toxics **11**(3): 291.

Amnuaylojaroen, T., N. Parasin and A. Limsakul (2022). "Health risk assessment of exposure near-future PM2.5 in Northern Thailand." Air Quality, Atmosphere & Health **15**(11): 1963-1979.

Berry, S. R. (2022). Estrogen as a therapeutic agent to protect against oxidative stress in aging and endocrine disruptors in humans and murine models, Northern Arizona University.

Bhatnagar, A. (2022). "Cardiovascular Effects of Particulate Air Pollution." Annu Rev Med **73**: 393-406.

Brook, R. D., S. Rajagopalan, C. A. Pope III, J. R. Brook, A. Bhatnagar, A. V. Diez-Roux, F. Holguin, Y. Hong, R. V. Luepker and M. A. Mittleman (2010). "Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association." Circulation **121**(21): 2331-2378.

Brucker, N., A. M. Moro, M. F. Charão, J. Durgante, F. Freitas, M. Baierle, S. Nascimento, B. Gauer, R. P. Bulcão and G. B. Bubols (2013). "Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers." Science of the total environment **463**: 884-893.

Campos, C., R. Guzmán, E. López-Fernández and Á. Casado (2011). "Evaluation of urinary biomarkers of oxidative/nitrosative stress in adolescents and adults with Down syndrome." Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease **1812**(7): 760-768.

Cui, X., J. Gong, H. Han, L. He, Y. Teng, T. Tetley, R. Sinharay, K. F. Chung, T. Islam and F. Gilliland (2018). "Relationship between free and total malondialdehyde, a well-established marker of oxidative stress, in various types of human biospecimens." Journal of thoracic disease **10**(5): 3088.

Fujitani, Y., A. Furuyama, M. Hayashi, H. Hagino and M. Kajino (2023). "Assessing oxidative stress induction ability and oxidative potential of PM2. 5 in cities in eastern and western Japan." Chemosphere **324**: 138308.

Glencross, D. A., T.-R. Ho, N. Camina, C. M. Hawrylowicz and P. E. Pfeffer (2020). "Air pollution and its effects on the immune system." Free Radical Biology and Medicine **151**: 56-68.

Il'yasova, D., P. Scarbrough and I. Spasojevic (2012). "Urinary biomarkers of oxidative status." Clinica chimica acta **413**(19-20): 1446-1453.

Jainonthee, C., Y.-L. Wang, C. W. Chen and K. Jainontee (2022). "Air pollution-related respiratory diseases and associated environmental factors in Chiang Mai, Thailand, in 2011–2020." Tropical Medicine and Infectious Disease **7**(11): 341.

Jarernwong, K., S. H. Gheewala and S. Sampattagul (2023). "Health Impact Related to Ambient Particulate Matter Exposure as a Spatial Health Risk Map Case Study in Chiang Mai, Thailand." Atmosphere **14**(2): 261.

Kho, Y., E.-H. Lee, H. J. Chae, K. Choi, D. Paek and S. Park (2015). "1-Hydroxypyrene and oxidative stress marker levels among painting workers and office workers at shipyard." International archives of occupational and environmental health **88**: 297-303.

Leni, Z., L. Künzi and M. Geiser (2020). "Air pollution causing oxidative stress." Current opinion in toxicology **20**: 1-8.

Li, N., T. Xia and A. E. Nel (2008). "The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles." Free radical biology and medicine **44**(9): 1689-1699.

Liu, C., Q. Liu, S. Song, W. Li, Y. Feng, X. Cong, Y. Ji and P. Li (2023). "The association between internal polycyclic aromatic hydrocarbons exposure and risk of Obesity—A systematic review with meta-analysis." Chemosphere **329**: 138669.

Liu, T., B. Jiang, B. Fu, C. Shang, H. Feng, T. Chen and Y. Jiang (2024). "PM2.5 Induces Cardiomyoblast Senescence via AhR-Mediated Oxidative Stress." Antioxidants **13**(7): 786.

Luo, K., I. Stepanov and S. S. Hecht (2019). "Chemical biomarkers of exposure and early damage from potentially carcinogenic airborne pollutants." Annals of Cancer Epidemiology **3**.

Makra, L., J. Puskás, I. Matyasovszky, Z. Csépe, E. Lelovics, B. Bálint and G. Tusnády (2015). "Weather elements, chemical air pollutants and airborne pollen influencing asthma emergency room visits in Szeged, Hungary: performance of two objective weather classifications." International journal of biometeorology **59**: 1269-1289.

Møller, P. and S. Loft (2010). "Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution." Environmental health perspectives **118**(8): 1126-1136.

Paesrivarotai, O. and G. Tanaksaranond (2021). Study of active fire evolution in northern Thailand using kernel density estimation (KDE). 26th National Convention on Civil Engineering.

Peretz, A., J. D. Kaufman, C. A. Trenga, J. Allen, C. Carlsten, M. R. Aulet, S. D. Adar and J. H. Sullivan (2008). "Effects of diesel exhaust inhalation on heart rate variability in human volunteers." Environmental research **107**(2): 178-184.

Schraufnagel, D. E., J. R. Balmes, C. T. Cowl, S. De Matteis, S.-H. Jung, K. Mortimer, R. Perez-Padilla, M. B. Rice, H. Riojas-Rodriguez and A. Sood (2019). "Air pollution and noncommunicable diseases: A review by the Forum of International Respiratory Societies' Environmental Committee, Part 2: Air pollution and organ systems." Chest **155**(2): 417-426.

Sirithian, D. and P. Thanatrakolsri (2022). "Relationships between Meteorological and Particulate Matter Concentrations (PM2.5 and PM10) during the Haze Period in Urban and Rural Areas, Northern Thailand." Air, Soil and Water Research **15**: 11786221221117264.

Sukkhum, S., A. Lim, T. Ingviya and R. Saelim (2022). "Seasonal patterns and trends of air pollution in the upper northern Thailand from 2004 to 2018." Aerosol and Air Quality Research **22**(5): 210318.

Supasri, T., S. H. Gheewala, R. Macatangay, A. Chakpor and S. Sedpho (2023). "Association between ambient air particulate matter and human health impacts in northern Thailand." Scientific Reports **13**(1): 12753.

Sutan, K., W. Naksen and T. Prapamontol (2017). "A simple high-performance liquid chromatography coupled to fluorescence detection method using column-switching technique for measuring urinary 1-hydroxypyrene from environmental exposure." tandem **15**: 16.

Tala, W., P. Kraisitnitikul and S. Chantara (2023). "Impact of Atmospheric Conditions and Source Identification of Gaseous Polycyclic Aromatic Hydrocarbons (PAHs) during a Smoke Haze Period in Upper Southeast Asia." Toxics **11**(12): 990.

Thongtip, S., P. Srivichai, N. Chaitiang and K. Tantrakarnapa (2022). "The influence of air pollution on disease and related health problems in Northern Thailand." Sains Malaysiana **51**(7): 1993-2002.

Toto, A., P. Wild, M. Graille, V. Turcu, C. Crézé, M. Hemmendinger, J.-J. Sauvain, E. Bergamaschi, I. Guseva Canu and N. B. Hopf (2022). "Urinary malondialdehyde (MDA) concentrations in the general population—a systematic literature review and meta-analysis." Toxics **10**(4): 160.

Viña, J., C. Borrás, J. Gambini, J. Sastre and F. V. Pallardó (2005). "Why females live longer than males: control of longevity by sex hormones." Science of Aging Knowledge Environment **2005**(23): pe17-pe17.

Wang, N., Y. Ma, Z. Liu, L. Liu, K. Yang, Y. Wei, Y. Liu, X. Chen, X. Sun and D. Wen (2019). "Hydroxytyrosol prevents PM2. 5-induced adiposity and insulin resistance by restraining

oxidative stress related NF-κB pathway and modulation of gut microbiota in a murine model." Free Radical Biology and Medicine **141**: 393-407.

Weary, T. E. (2023). A One Health Approach to Respiratory Disease in Children and Chimpanzees in Uganda, The University of Wisconsin-Madison.

Zhang, H., D. Il'yasova, J. Sztaray, S. P. Young, F. Wang and D. S. Millington (2010). "Quantification of the oxidative damage biomarker 2, 3-dinor-8-isoprostaglandin-F2α in human urine using liquid chromatography–tandem mass spectrometry." Analytical biochemistry **399**(2): 302-304.

Zhang, H., R. Liu, L. Yang, H. Cheng, S. Wang, B. Zhang, J. Shao, S. Ma, D. Norbäck and X. Zhang (2023). "Exposure to polycyclic aromatic hydrocarbons (PAHs) in outdoor air and respiratory health, inflammation and oxidative stress biomarkers: a panel study in healthy young adults." Science of the Total Environment **899**: 165582.

Zhao, X., H. Xu, Y. Li, Y. Liu, C. Guo and Y. Li (2023). "Status and frontier analysis of indoor PM2. 5-related health effects: a bibliometric analysis." Reviews on Environmental Health(0).

Zhao, Z., W. Gong, L. Liu, M. Wang, Z. Tang, N. Mohammadtursun, L. Lu, J. Liu, M. Li and Y. Lv (2018). "Urinary 8-hydroxydeoxyguanosine is a better biomarker of aging in non-smokers." Traditional Medicine and Modern Medicine **1**(01): 43-51.

Figure 1

Mechanism of oxidative stress induced by PM2.5 exposure, showing the imbalance between ROS and antioxidants leading to oxidative damage.

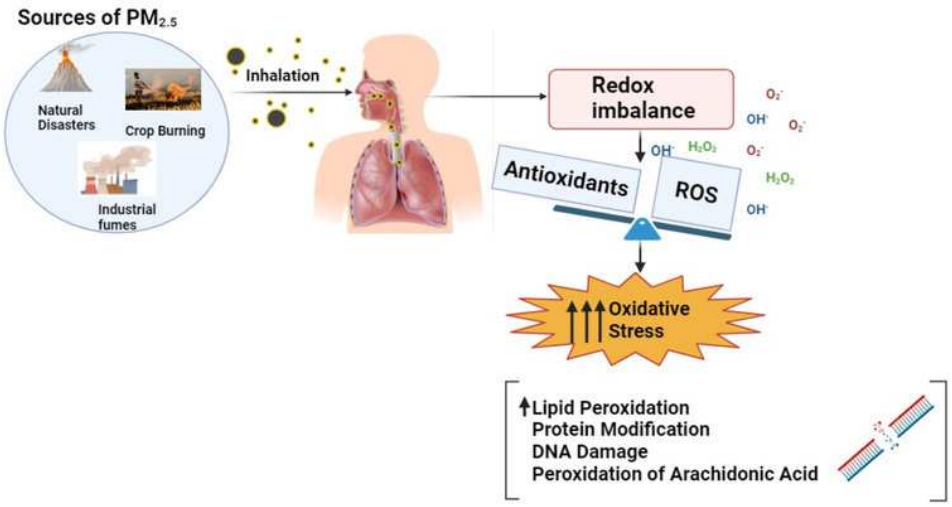


Figure 1 Mechanism of oxidative stress induced by PM2.5 exposure, showing the imbalance between ROS and antioxidants leading to oxidative damage.

Figure 2

Map showing the study area in Chiang Mai, Thailand.

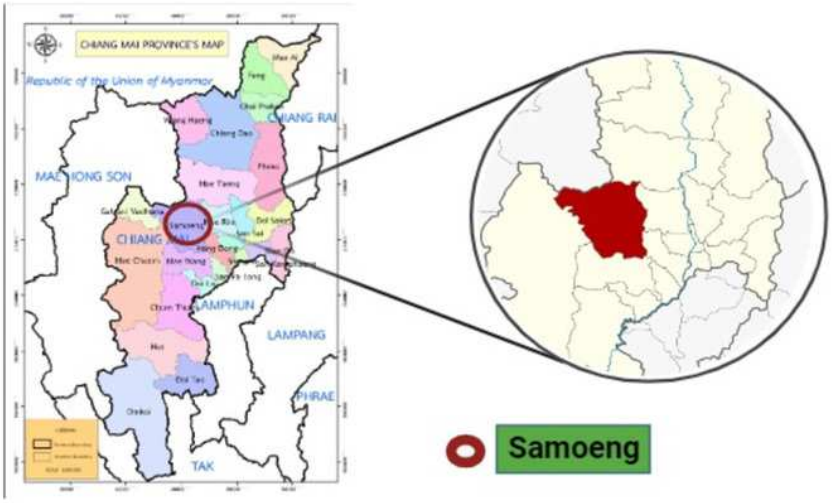


Figure 2 Map showing the study area in Chiang Mai, Thailand.

Figure 3

Mean concentrations of PM2.5 (µg/m³) recorded during high (March-April 2023) and low (May-July 2023) pollution seasons.

The error bars represent the standard error of the mean (SEM).

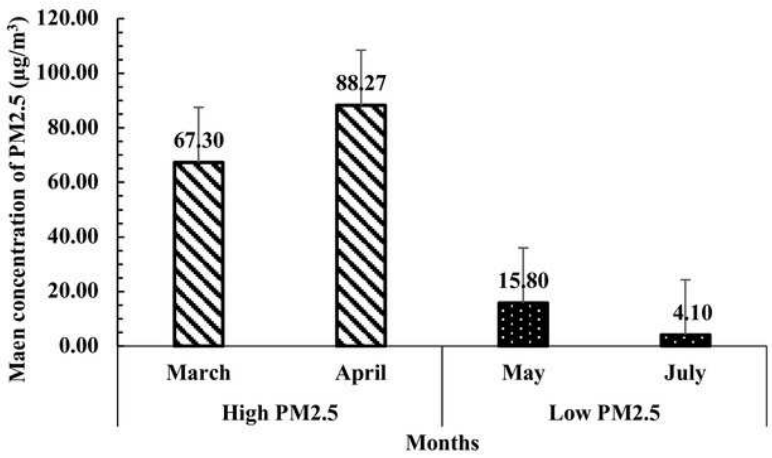


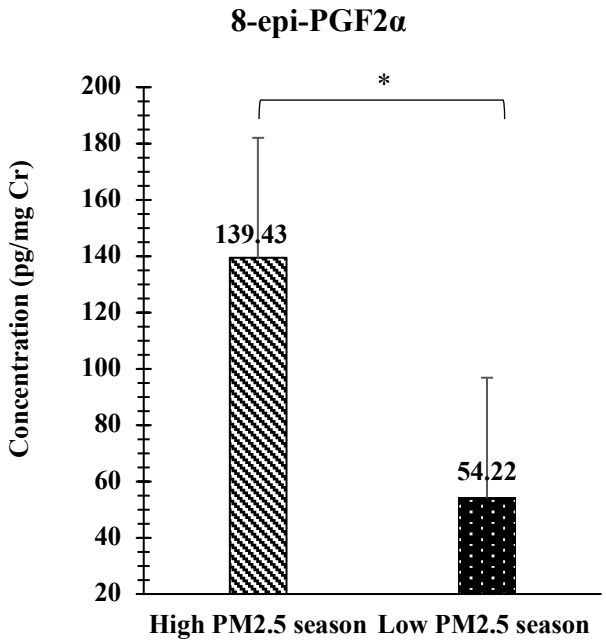
Figure 3 Mean concentrations of PM2.5 (µg/m³) recorded during high (March-April 2023) and low (May-July 2023) pollution seasons. The error bars represent the standard error of the mean (SEM).

Figure 4(on next page)

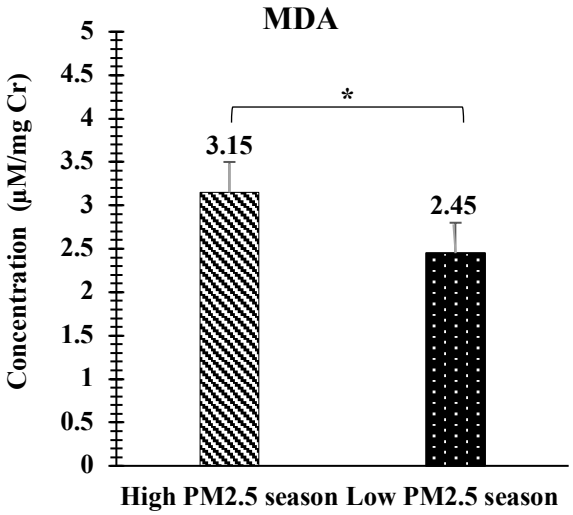
Comparison of urinary concentrations of oxidative stress biomarkers, including A) 8-epi-PGF2α, B) Malondialdehyde (MDA), and C) 1-hydroxypyrene (1-OHP) between high PM2.5 and low PM2.5 seasons.

Data are presented as median values with interquartile ranges (Q1, Q3) for each biomarker. The Wilcoxon signed-rank test was used to assess the significance of differences between seasons, with all *p-values < 0.05 indicating statistically significant increases in biomarker levels during the high PM2.5 season.

A)


8-epi-PGF2α
Concentration (pg/mg Cr)
200
180
160
140
120
100
80
60
40
20
*
139.43
54.22
High PM2.5 season
Low PM2.5 season

B)


MDA
Concentration (µM/mg Cr)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
3.15
2.45
High PM2.5 season
Low PM2.5 season

C)

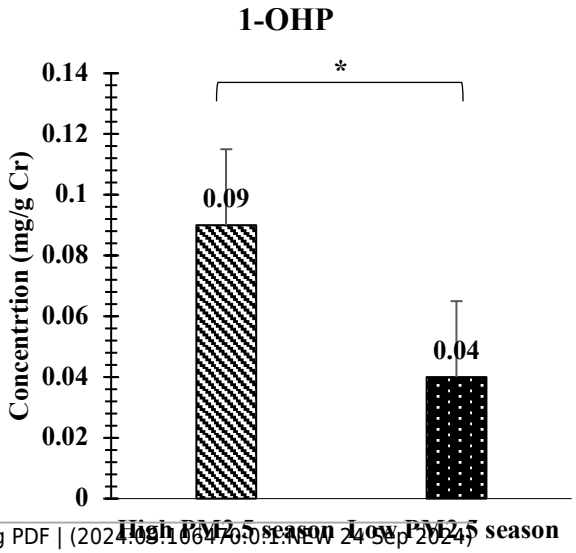

1-OHP
Concentrtion (mg/g Cr)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
*
0.09
0.04
High PM2.5 season
Low PM2.5 season

Figure 4 Comparison of urinary concentrations of oxidative stress biomarkers, including A) 8-epi-PGF2α, B) Malondialdehyde (MDA), and C) 1-hydroxypyrene (1-OHP) between high PM2.5 and low PM2.5 seasons. Data are presented as median values with interquartile ranges (Q1, Q3) for each biomarker. The Wilcoxon signed-rank test was used to assess the significance of differences between seasons, with all *p-values < 0.05 indicating statistically significant increases in biomarker levels during the high PM2.5 season.

Table 1(on next page)

Baseline characteristics of the participants (N = 25).

Table 1 Baseline characteristics of the participants (N = 25).

Characteristic	N (%)
Age (years)	
Mean ±SD	48.1±14.6
Gender	
Male	10 (40.0%)
Female	15 (60.0%)
Smoking	
Yes	21 (84.0%)
No	4 (16.0%)
Alcohol Consumption	
Yes	6 (24.0%)
No	19 (76.0%)
Education Level	
Less than high school	11 (44.0%)
High school	3 (12.0%)
College/University	11 (44.0%)
Occupation	
Agriculture	13 (52.0%)
Service	3 (12.0%)
Others	9 (36.0%)

Table 2(on next page)

Multivariate regression analysis shows significant associations between PM2.5 exposure and elevated levels of urinary oxidative stress biomarkers, even after adjusting for age, gender, and smoking status.

1-OHP: 1-hydroxypyrene; MDA: Malondialdehyde; 8-iso-PGF2α: 8-iso-prostaglandin-F2α.

*P-values < 0.05 indicating statistically significant association.

Table 2 Multivariate regression analysis shows significant associations between PM2.5 exposure and elevated levels of urinary oxidative stress biomarkers, even after adjusting for age, gender, and smoking status.

Dependent Variable	Independent Variables	Coefficient (Exp(b))	Standard Error	z-value	p-Value	95% CI
1-OHP	PM2.5	1.014	0.003	4.25	<0.01*	1.01,1.02
	Age	0.992	0.01	-0.80	0.422	0.97,1.01
	Gender (Female)	1.03	0.33	0.08	0.933	0.55,1.91
	Smoking Status (Smoker)	0.77	0.33	-0.61	0.543	0.33,1.80
MDA	PM2.5	0.998	0.0008	-1.95	0.051	0.997,1.00
	Age	1.01	0.002	2.70	0.007	1.00,1.01
	Gender (Female)	1.15	0.079	2.04	0.041	1.01,1.32
	Smoking Status (Smoker vs Non-Smoker)	1.05	0.10	0.55	0.583	0.87,1.27
8-iso-PGF2α	PM2.5	1.008	0.004	2.00	0.045*	1.00,1.02
	Age	1.013	0.014	1.29	0.196	0.99,1.03
	Gender (Female)	1.377	0.465	0.95	0.344	0.71,2.67
	Smoking Status (Smoker)	2.22	1.017	1.75	0.081	0.91,5.45

1-OHP: 1-hydroxypyrene; MDA: Malondialdehyde; 8-iso-PGF2α: 8-iso-prostaglandin-F2α.

*P-values < 0.05 indicating statistically significant association.