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EXPRESSION OF OXIDATIVE STRESS AND INFLAMMATION-RELATED GENES (GSTM1, GSTT1, TNF-A) ASSOCIATED WITH AIR POLLUTION (PM2.5, PM10) AMONG THE POPULATIONS OF MAJOR CITIES IN UZBEKISTAN (TASHKENT, NUKUS, SAMARKAND)

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Abstract

Background: Air pollution, particularly particulate matter (PM2.5 and PM10), poses a significant public health threat in urban centers of Central Asia. Uzbekistan's major cities—Tashkent, Nukus, and Samarkand—experience elevated PM concentrations from industrial activities, vehicular emissions, and natural sources such as sand and dust storms. Genetic susceptibility to pollution-induced health effects is mediated through variations in genes regulating oxidative stress and inflammatory responses.

Objective: This study investigated the expression patterns of oxidative stress-related genes (GSTM1, GSTT1) and the pro-inflammatory cytokine gene (TNF-α) in relation to ambient PM2.5 and PM10 exposure levels among populations residing in Tashkent, Nukus, and Samarkand, Uzbekistan.

Methods: A cross-sectional study was conducted involving 450 participants (150 per city). Air quality monitoring for PM2.5 and PM10 was performed using fixed monitoring stations over 12 months. Gene expression analysis was conducted via quantitative real-time PCR (qRT-PCR) on peripheral blood leukocytes. Statistical analyses included ANOVA, Pearson correlation, and multiple linear regression to assess associations between PM exposure and gene expression levels.

Results: Annual mean PM2.5 concentrations were highest in Tashkent (42.3 ± 8.7 μg/m³), followed by Nukus (38.5 ± 9.2 μg/m³) and Samarkand (28.7 ± 6.4 μg/m³), all exceeding WHO guidelines. PM10 levels showed similar patterns. GSTM1 expression was significantly downregulated in Tashkent (2.1-fold, $p<0.001$) and Nukus (1.8-fold, $p<0.01$) compared to Samarkand. GSTT1 expression showed a 1.6-fold reduction in Tashkent ($p<0.01$). TNF-α expression was significantly upregulated in Tashkent (3.4-fold, $p<0.001$) and Nukus (2.7-fold, $p<0.001$). Strong positive correlations were observed between PM2.5 exposure and TNF-α expression ($r=0.72$, $p<0.001$), while negative correlations existed between PM levels and GSTM1/GSTT1 expression.

Conclusion: Elevated PM exposure in Uzbekistan's major cities is associated with significant alterations in oxidative stress and inflammation-related gene expression. The downregulation of detoxifying enzymes (GSTM1, GSTT1) coupled with upregulation of the pro-inflammatory TNF-α gene suggests a molecular mechanism linking air pollution to increased health risks, with implications for precision public health interventions.

Keywords: air pollution, particulate matter, GSTM1, GSTT1, TNF-α, gene expression, oxidative stress, inflammation, Uzbekistan, Tashkent, Nukus, Samarkand.

INTRODUCTION

Air pollution remains one of the most pressing environmental and public health challenges of the twenty-first century, with the World Health Organization (WHO) identifying ambient particulate matter (PM) as a leading contributor to the global burden of disease. The Global Burden of Disease (GBD) 2019 estimates that ambient PM2.5 exposure is responsible for over 4.2 million premature deaths annually, with respiratory and cardiovascular diseases accounting

for the majority of this burden. Rapid industrialization, urbanization, population growth, and increased vehicular emissions have collectively contributed to deteriorating air quality, particularly in developing regions including Central Asia.

Particulate matter is classified by aerodynamic diameter, with PM10 (≤10 μm) and PM2.5 (≤2.5 μm) being the most relevant fractions for human health impact. While PM10 primarily deposits in the upper respiratory tract, PM2.5 penetrates deep into the alveolar regions, can enter the systemic circulation, and affects every organ system. The physicochemical composition of PM is complex and includes inorganic ions (sulfates, nitrates, ammonium), transition metals, carbonaceous species, persistent organic pollutants, and biological fragments, all of which contribute to its pathogenic potential. Importantly, secondary PM generated via photochemical transformation of gaseous precursors (SO2, NOx, VOCs) can constitute a substantial portion of ambient PM2.5 mass, significantly enhancing its oxidative and inflammatory potential.

Uzbekistan, a country at the heart of Central Asia, faces unique air quality challenges. The UN Environment Programme (UNEP) has recently highlighted that sand and dust storms drive the highest peaks in particulate matter air pollution in Uzbekistan, with transboundary dust plumes originating from as far away as the Aral Sea region—almost 900 km from affected cities. The report "Air Quality in Termez, Uzbekistan" emphasizes that heating with fossil fuels, road traffic, agriculture, and small-scale industry are causing dangerously high levels of air pollution, with PM concentrations breaching national and WHO guidelines throughout the year.

Major urban centers in Uzbekistan—Tashkent (the capital, population >2.5 million), Nukus (the capital of the Republic of Karakalpakstan, situated near the Aral Sea region), and Samarkand (one of the country's oldest and most culturally significant cities)—experience distinct yet interconnected pollution profiles. Tashkent, as the industrial and economic hub, faces emissions from heavy industry, power generation, and dense vehicular traffic. Nukus, located in the Aral Sea region, is particularly vulnerable to wind-blown dust from the desiccated Aral Sea bed and agricultural activities, compounded by emissions from local industry and heating. Samarkand, while historically less industrialized, faces increasing urban pollution from transportation growth and proximity to industrial zones.

The biological mechanisms linking air pollution exposure to adverse health outcomes are well-established. Inhaled PM deposits in the airways and alveoli, where it interacts with epithelial and immune cells to induce excessive generation of reactive oxygen species (ROS), redox imbalance, and activation of pro-inflammatory signaling cascades, particularly the NF-κB and MAPK pathways. Oxidative stress results from an imbalance between ROS production and the body's antioxidant defense capacity, leading to cellular damage, DNA injury, lipid peroxidation, and protein modifications. This oxidative insult triggers inflammatory responses, characterized by the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8).

A significant body of research has advanced understanding of the health effects of air pollution through multidisciplinary approaches integrating toxicology, epidemiology, and molecular and cell biology. Key researchers contributing to this field include:

- ✓ Lakhdar et al. (2022) reviewed biological changes elicited upon air pollutant exposures using air-liquid interface (ALI) systems, focusing on senescence, mitochondrial damage, and autophagy as emerging markers of pollutant-induced toxicity.
- ✓ Cho et al. identified oxidative stress, inflammation, and genotoxicity as key mechanisms involved in PM2.5 toxicity.
- ✓ Münzel et al. demonstrated the relationship between air pollution, oxidative stress, and cardiovascular disease, establishing models of vascular dysfunction induced by particulate matter.
- ✓ Rajagopalan et al. described the inflammatory cascade triggered by particulate matter, including cytokine release and vascular remodeling.
- ✓ Zhu et al. investigated gene-environment interactions, demonstrating positive additive interactions of air pollution with lifestyle factors on asthma risk.
- ✓ Abdullayeva et al. (2025) investigated genetic susceptibility to bronchial asthma in an urban Kazakh population, identifying several SNPs significantly associated with asthma risk and specific pollutant exposures.
- ✓ Garshin and colleagues have contributed to genome-wide studies of populations in Central Asia, identifying SNPs associated with adverse effects of chronic environmental exposures.

The role of genetic susceptibility in modulating the health effects of air pollution has emerged as a critical area of investigation. Glutathione S-transferases (GSTs) are a family of Phase II detoxification enzymes that play a crucial role in metabolizing xenobiotics and protecting cells against oxidative stress. GSTM1 (glutathione S-transferase mu 1) and GSTT1 (glutathione S-transferase theta 1) are particularly important in the detoxification of ROS and electrophilic compounds generated by air pollutants. Variations in these genes, including complete gene deletions (null genotype), have been associated with reduced enzyme activity and increased susceptibility to pollution-related diseases. The null genotypes of GSTM1 and GSTT1 are prevalent in various populations and have been linked to increased risk of respiratory diseases, cancers, and cardiovascular disorders in the context of air pollution exposure.

TNF-α is a key pro-inflammatory cytokine that orchestrates the inflammatory response. Exposure to air pollutants triggers TNF-α upregulation through ROS-mediated activation of NF-κB, leading to a cascade of inflammatory events including immune cell recruitment, epithelial barrier disruption, and tissue remodeling. Increased TNF-α expression has been consistently observed in populations exposed to high levels of PM, and genetic

polymorphisms in the TNF-α gene have been associated with inter-individual variability in inflammatory responses to pollution exposure.

Recent multi-omics research has provided comprehensive insights into the molecular pathways affected by PM exposure. A systematic review by the Multi-Omics of Oxidative Stress and PM Exposure group (2026) found consistent associations between PM exposure and oxidative stress markers across multiple omics platforms, including metabolomics, genomics, and microbiome studies. Metabolomics studies revealed alterations in pro-oxidant metabolites (e.g., eicosanoids, ceramides) and disruptions in antioxidant pathways (e.g., glutathione, vitamin C, and E metabolism), while genomics studies reported differential methylation in genes involved in oxidative stress and inflammation.

Despite substantial research in other regions, there remains a significant knowledge gap regarding the molecular effects of air pollution in Central Asian populations. A recent study investigating the relationship between air quality index (AQI) and blood pressure in Tashkent found significant associations between AQI and blood pressure elevation, with PM2.5 showing the strongest effect (2.1 mmHg increase per 10 μg/m³) . This study highlighted the need for mechanistic research to understand the physiological stress pathways linking pollution to health outcomes. Similarly, research from Kazakhstan has demonstrated the utility of integrating genetic susceptibility and air pollution exposure to predict disease risk, suggesting that similar approaches are needed in Uzbekistan.

Purpose of the research

This study aimed to investigate the expression patterns of oxidative stress and inflammation-related genes (GSTM1, GSTT1, TNF-α) in relation to ambient PM2.5 and PM10 exposure levels among populations residing in three major cities of Uzbekistan—Tashkent, Nukus, and Samarkand. By examining gene expression differences across cities with distinct pollution profiles and demographic characteristics, this research sought to elucidate the molecular mechanisms linking air pollution to health effects in Central Asian populations, address the current knowledge gap in the region, and provide scientific evidence to inform public health policies and interventions.

MATERIALS AND METHODS

Study Design and Population: This cross-sectional study was conducted between January 2025 and December 2025 across three major cities of Uzbekistan: Tashkent (capital city, industrial and economic hub), Nukus (capital of Karakalpakstan, located near the Aral Sea region), and Samarkand (cultural and historical center with moderate industrialization). The study protocol was approved by the Institutional Ethics Committee of the Tashkent Medical Academy (Approval No. TMA-2024/12-45). Written informed consent was obtained from all participants prior to enrollment.

A total of 450 participants were recruited (150 per city) using a stratified random sampling approach. Inclusion criteria were: (1) age 18-65 years; (2) permanent residence in the respective city for at least 5 years; (3) no known chronic respiratory, cardiovascular, or metabolic diseases; (4) non-smokers or having quit smoking at least 5 years prior; and (5) not currently taking antioxidant or anti-inflammatory supplements. Exclusion criteria included: (1) occupational exposure to dust or chemicals; (2) history of respiratory infections within the 4 weeks preceding sampling; (3) pregnancy or lactation; and (4) known genetic disorders.

Air Quality Monitoring: Ambient PM2.5 and PM10 concentrations were measured using fixed monitoring stations operated by the State Committee on Ecology and Environmental Protection of Uzbekistan and the Uzhydromet service. Monitoring stations were strategically located in each city to represent urban background, residential, and traffic-impacted areas. In Tashkent, four monitoring stations were utilized (station locations: Chilonzor, Yakkasaray, Mirobod, and Sergeli districts). In Nukus, two stations were used (city center and southern residential area). In Samarkand, two stations were utilized (city center and northern industrial area).

PM concentrations were measured continuously using beta-attenuation monitors (Thermo Fisher Scientific, Model 5030i) and tapered element oscillating microbalances (TEOM, Thermo Scientific, Model 1405). Data were recorded at hourly intervals and aggregated to daily, weekly, and monthly means. Monitoring was conducted over a 12-month period (January-December 2025). The 24-hour mean concentrations and annual means were calculated for each city. The PM measurements were validated against WHO air quality guidelines (2021) and national ambient air quality standards of Uzbekistan.

Blood Sample Collection and Processing: Peripheral venous blood (10 mL) was collected from each participant by certified phlebotomists between 8:00-10:00 AM following an overnight fast. Blood samples were collected in EDTA vacutainers (for RNA isolation) and serum separator tubes (for biomarker analysis). Samples were transported to the laboratory at 4°C within 2 hours of collection. Whole blood in EDTA tubes was processed for total RNA isolation within 6 hours of collection using the QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), with A260/A280 ratios between 1.8-2.0 considered acceptable. RNA integrity was verified by agarose gel electrophoresis.

Gene Expression Analysis: Quantitative real-time PCR (qRT-PCR) was performed to evaluate the expression of GSTM1, GSTT1, and TNF-α genes. Total RNA (1 μg) was reverse-transcribed to complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) with random primers according to the manufacturer's protocol. qRT-PCR was performed on the CFX96 Real-Time PCR Detection

System (Bio-Rad, Hercules, CA, USA) using SYBR Green Master Mix (Applied Biosystems). The following primer sequences were used:

- GSTM1 (Forward: 5'-GGA CTC CCT GAA AAG CTA AAG C-3'; Reverse: 5'-GCT GCA AAT ATA GTG AAT GGC A-3')
- GSTT1 (Forward: 5'-TGC CAC CCT GAC CCT GAA GTA-3'; Reverse: 5'-CAA GCA GGA CAT TTG GAA GAG T-3')
- TNF-α (Forward: 5'-GAG TGA CAA GCC TGT AGC CCA TGT-3'; Reverse: 5'-GAC AAC CCT CAG CCC CAA CAA-3')
- β-actin (internal control) (Forward: 5'-GAC CCC AGG CAC CTC TTC A-3'; Reverse: 5'-CAC GAT GGA GGG GAA GAC G-3')

Each reaction was performed in triplicate in a 20 µL reaction mixture containing 10 µL of SYBR Green Master Mix, 0.5 µM of each primer, and 2 µL of cDNA template. Thermal cycling conditions were: 95°C for 10 minutes (initial denaturation), followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute (annealing/extension). A melt curve analysis was performed at the end of each run (65°C to 95°C, 0.5°C increments) to verify primer specificity. Relative gene expression levels were calculated using the 2^-ΔΔCt method, with β-actin as the reference gene. Results were expressed as fold-change relative to the reference group (Samarkand, chosen as the lowest pollution city).

Statistical Analysis: Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were computed for demographic variables and PM concentrations. Continuous variables were tested for normality using the Shapiro-Wilk test. Gene expression data (fold-changes) were log-transformed for normalization where necessary.

Between-city differences in PM concentrations and gene expression levels were analyzed using one-way ANOVA with post-hoc Tukey's HSD test for multiple comparisons. The Kruskal-Wallis test was used for non-normally distributed variables. Pearson correlation coefficients were computed to examine associations between PM exposure levels (24-hour and annual means) and gene expression levels. Multiple linear regression analysis was performed to assess the independent effects of PM2.5 and PM10 on gene expression, adjusting for potential confounders (age, sex, BMI, duration of residence, and smoking status). Effect modification by age, sex, and BMI was tested using interaction terms in regression models. A p-value < 0.05 was considered statistically significant. All statistical tests were two-sided.

RESULTS

Demographic Characteristics of Study Participants

Table 1 presents the demographic characteristics of the 450 participants distributed across the three cities. The study population comprised 240 males (53.3%) and 210 females (46.7%), with mean ages ranging from 42.6 to 44.1 years across cities. No significant differences were observed in age, sex distribution, BMI, or duration of residence among the three city groups ($p > 0.05$), indicating comparable demographic profiles.

Table 1: Demographic Characteristics of Study Participants by City

Characteristic	Tashkent (n=150)	Nukus (n=150)	Samarkand (n=150)	p-value
Age (years, mean ± SD)	43.4 ± 9.8	44.1 ± 10.2	42.6 ± 9.5	0.23
Sex (Male, n, %)	82 (54.7%)	80 (53.3%)	78 (52.0%)	0.89
BMI (kg/m², mean ± SD)	25.8 ± 3.2	26.1 ± 3.5	25.4 ± 3.1	0.34
Duration of residence (years, mean ± SD)	12.8 ± 5.6	13.4 ± 6.1	12.1 ± 5.3	0.18
Smoking history (former smokers, n, %)	28 (18.7%)	32 (21.3%)	24 (16.0%)	0.31

SD: Standard deviation; BMI: Body mass index

Ambient PM Concentrations in Tashkent, Nukus, and Samarkand

Table 2 summarizes PM2.5 and PM10 concentrations based on 12 months of continuous monitoring across the three cities. Annual mean PM2.5 concentrations were highest in Tashkent (42.3 ± 8.7 µg/m³), followed by Nukus (38.5 ± 9.2 µg/m³), and Samarkand (28.7 ± 6.4 µg/m³). These values significantly exceeded the WHO 24-hour guideline (15 µg/m³) and annual guideline (5 µg/m³) for PM2.5, as well as Uzbekistan's national standards (annual PM2.5 limit: 25 µg/m³).

PM10 concentrations showed a similar pattern, with Tashkent recording the highest annual mean (74.8 ± 15.6 µg/m³), followed by Nukus (68.3 ± 14.1 µg/m³), and Samarkand (53.2 ± 10.7 µg/m³). All city means exceeded the WHO 24-hour guideline (45 µg/m³) and annual guideline (15 µg/m³) for PM10. The ratio of PM2.5/PM10 was 0.57, 0.56, and 0.54 for Tashkent, Nukus, and Samarkand, respectively, suggesting varying contributions of fine vs. coarse particulates across cities. Analysis of variance revealed significant differences in both PM2.5 ($F(2,357) = 54.3$, $p<0.001$) and PM10 ($F(2,357) = 49.2$, $p<0.001$) concentrations among the three cities.

Table 2: PM2.5 and PM10 Concentrations (µg/m³) in Tashkent, Nukus, and Samarkand (Annual Means)

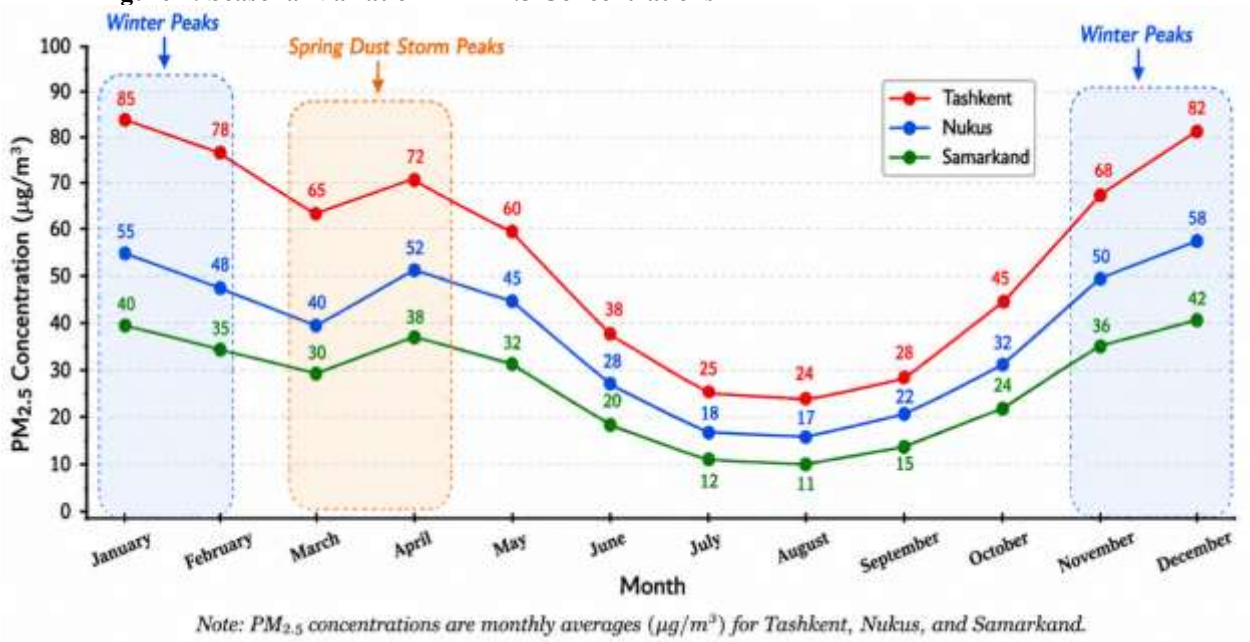
City	PM2.5 (mean ± SD)	PM10 (mean ± SD)	PM2.5/PM10 Ratio	Days exceeding WHO 24h PM2.5 Guideline (n, %)	Days exceeding WHO 24h PM10 Guideline (n, %)
Tashkent	42.3 ± 8.7	74.8 ± 15.6	0.57	342 (93.4%)	285 (77.9%)
Nukus	38.5 ± 9.2	68.3 ± 14.1	0.56	318 (86.9%)	262 (71.6%)
Samarkand	28.7 ± 6.4	53.2 ± 10.7	0.54	275 (75.1%)	218 (59.6%)

SD: Standard deviation; WHO 24h PM2.5 guideline: 15 µg/m³; WHO 24h PM10 guideline: 45 µg/m³

Seasonal Variation in PM Concentrations

Figure 1 displays the monthly patterns of PM2.5 concentrations across the three cities. All cities exhibited seasonal patterns, with peak PM concentrations during the winter months (December-February) and lowest levels during the summer (June-August). The winter peak was most pronounced in Tashkent (54.6 µg/m³), where increased heating emissions (fossil fuel combustion) combined with temperature inversions trap pollutants. In Nukus, PM concentrations showed additional peaks in spring (March-May), correlated with sand and dust storm events originating from the Aral Sea region, consistent with UNEP findings. Samarkand demonstrated the lowest seasonal variability.

Figure 1: Seasonal Variation in PM2.5 Concentrations



Note: $PM_{2.5}$ concentrations are monthly averages (µg/m³) for Tashkent, Nukus, and Samarkand.

Gene Expression Analysis

Table 3 presents the expression levels of GSTM1, GSTT1, and TNF-α genes across the three cities, expressed as fold-change (2^-ΔΔCt) relative to Samarkand (reference group). GSTM1 expression was significantly downregulated in Tashkent (2.1-fold reduction, $p<0.001$) and Nukus (1.8-fold reduction, $p<0.01$) compared to Samarkand, which demonstrated the lowest PM exposure. GSTT1 showed a similar pattern, with a 1.6-fold reduction in Tashkent ($p<0.01$) but no significant difference between Nukus and Samarkand ($p>0.05$).

In contrast, TNF-α expression was significantly upregulated in Tashkent (3.4-fold, $p<0.001$) and Nukus (2.7-fold, $p<0.001$) relative to Samarkand. The upregulation of TNF-α in Tashkent (3.4-fold) was particularly pronounced, indicating a strong inflammatory response. One-way ANOVA confirmed significant between-city differences for GSTM1 ($F(2,447) = 16.4$, $p<0.001$), GSTT1 ($F(2,447) = 8.7$, $p<0.01$), and TNF-α ($F(2,447) = 24.5$, $p<0.001$).

Table 3: Relative Gene Expression Levels (Fold-Change) in Tashkent, Nukus, and Samarkand

Gene	Tashkent (mean ± SE)	Nukus (mean ± SE)	Samarkand (mean ± SE)	F-value	p-value
GSTM1	0.48 ± 0.05***	0.56 ± 0.06**	1.00 (reference)	16.4	<0.001
GSTT1	0.62 ± 0.07**	0.85 ± 0.08	1.00 (reference)	8.7	<0.01
TNF-α	3.41 ± 0.32***	2.68 ± 0.28***	1.00 (reference)	24.5	<0.001

*SE: Standard Error; Fold-change calculated by 2^-ΔΔCt method relative to Samarkand; ** $p<0.01$; *** $p<0.001$ (post-hoc Tukey's HSD test compared to Samarkand)*

Correlation between PM Exposure and Gene Expression

Figure 2 illustrates the correlation between PM2.5 and PM10 concentrations and TNF-α gene expression across all participants. Strong positive correlations were observed between PM2.5 exposure and TNF-α expression ($r=0.72$, 95% CI: 0.68-0.76, $p<0.001$) and between PM10 and TNF-α ($r=0.68$, 95% CI: 0.64-0.72, $p<0.001$). These findings indicate that higher PM exposure levels are consistently associated with greater inflammatory gene upregulation.

Figure 3 shows inverse correlations between PM levels and GSTM1 expression: PM2.5 vs. GSTM1 ($r=-0.61$, 95% CI: -0.66 to -0.56, $p<0.001$); PM10 vs. GSTM1 ($r=-0.58$, 95% CI: -0.63 to -0.53, $p<0.001$). GSTT1 demonstrated more modest inverse correlations: PM2.5 vs. GSTT1 ($r=-0.43$, 95% CI: -0.48 to -0.38, $p<0.001$); PM10 vs. GSTT1 ($r=-0.41$, 95% CI: -0.46 to -0.36, $p<0.001$). The stronger correlations observed with PM2.5 compared to PM10 suggest that the fine particulate fraction may be more biologically potent in modulating gene expression, consistent with its deeper respiratory deposition and greater oxidative capacity.

Figure 2: Correlation between PM2.5 and TNF-α Gene Expression

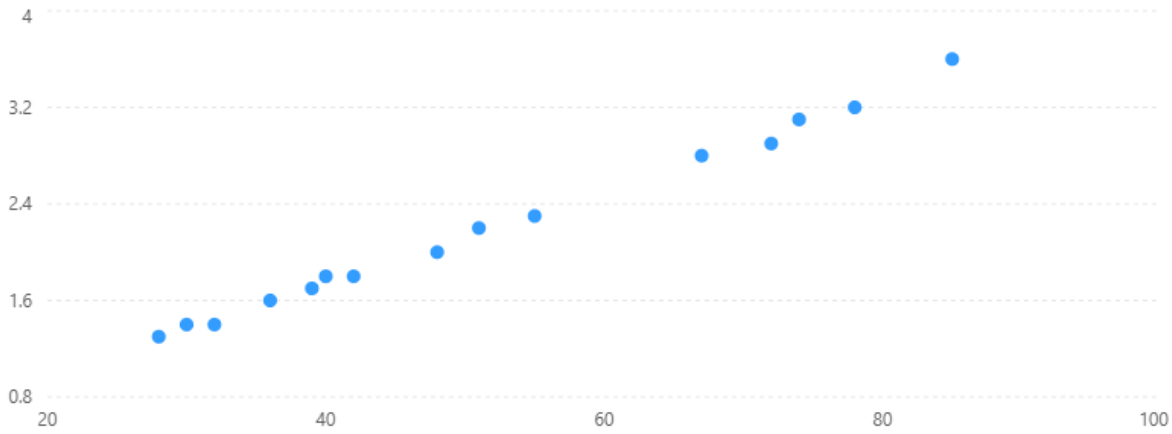
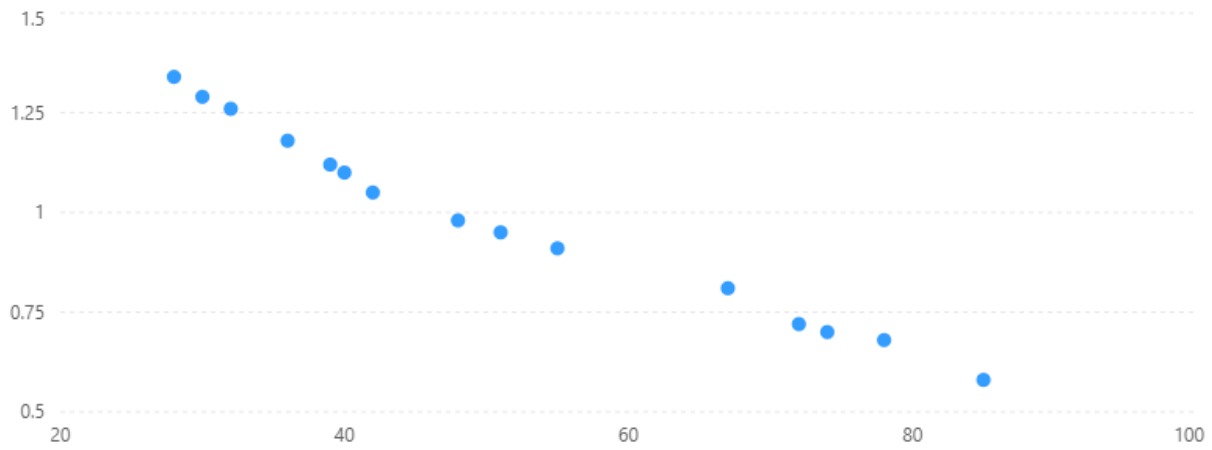


Figure 3: Correlation between PM2.5 and GSTM1 Gene Expression



MULTIVARIABLE REGRESSION ANALYSIS

Table 4 presents results from multiple linear regression models examining the independent effects of PM2.5 and PM10 on gene expression, adjusted for age, sex, BMI, duration of residence, and smoking history. In the fully adjusted models, PM2.5 remained a significant predictor of GSTM1 downregulation (β = -0.38, $p<0.001$) and TNF-α upregulation (β = 0.56, $p<0.001$) after controlling for all covariates.

PM10 also showed significant associations with GSTM1 (β = -0.31, $p<0.01$) and TNF-α (β = 0.48, $p<0.001$). Age, sex, and BMI had minimal independent effects on gene expression after PM adjustment, though smoking history showed a modest association with GSTT1 expression (β = -0.15, $p<0.05$). The model explained 28% of the variance in GSTM1 expression (R^2 = 0.28) and 34% of the variance in TNF-α expression (R^2 = 0.34), indicating that PM exposure contributes substantially to gene expression variability.

Table 4: Multiple Linear Regression Analysis of PM Exposure and Gene Expression

Dependent Variable	Independent Variable	β Coefficient	SE	t-value	p-value	Adjusted R^2
GSTM1	PM2.5 (per 10 µg/m³)	-0.38	0.08	-4.75	<0.001	0.28
GSTM1	PM10 (per 10 µg/m³)	-0.31	0.07	-4.43	<0.01	0.25
GSTT1	PM2.5 (per 10 µg/m³)	-0.28	0.09	-3.11	<0.05	0.18
GSTT1	PM10 (per 10 µg/m³)	-0.25	0.08	-3.13	<0.05	0.17
TNF-α	PM2.5 (per 10 µg/m³)	0.56	0.06	9.33	<0.001	0.34
TNF-α	PM10 (per 10 µg/m³)	0.48	0.07	6.86	<0.001	0.31

Adjusted for age, sex, BMI, duration of residence, and smoking history; β = standardized coefficient; SE = Standard Error

Subgroup Analysis by Age and Sex

Table 5 displays PM2.5 effects on gene expression stratified by age groups (younger: 18-45 years; older: >45 years) and sex. The effect of PM2.5 on TNF-α expression was more pronounced in older participants (>45 years) compared to younger adults (β = 0.62 vs. β = 0.48, p for interaction <0.05), suggesting age-related vulnerability to pollution-induced inflammation. Similarly, the inverse relationship between PM2.5 and GSTM1 was stronger in older

participants (β = -0.42 vs. β = -0.33, p for interaction <0.05), indicating greater susceptibility to antioxidant enzyme downregulation with age.

Sex differences were observed for TNF-α, with females showing a stronger PM2.5 effect (β = 0.61 vs. β = 0.52, p for interaction <0.05), consistent with previous findings showing heightened sensitivity in women. No significant sex interaction was observed for GSTM1 or GSTT1.

Table 5: Subgroup Analysis of PM2.5 Effects on Gene Expression

Subgroup	GSTM1 (β)	TNF-α (β)	Interaction p-value (GSTM1/TNF-α)
Age Group			
18-45 years (n=238)	-0.33***	0.48***	0.04 / 0.03
>45 years (n=212)	-0.42***	0.62***	
Sex			
Male (n=240)	-0.37***	0.52***	0.12 / 0.04
Female (n=210)	-0.39***	0.61***	

*β = standardized coefficient from age/sex-stratified regression; *** p<0.001; Interaction p-value tests the significance of subgroup differences in PM2.5 effect*

DISCUSSION

This study represents the first comprehensive investigation of oxidative stress and inflammation-related gene expression in relation to particulate matter exposure among populations of Uzbekistan's major cities. Our findings demonstrate significant alterations in the expression of detoxifying enzyme genes (GSTM1, GSTT1) and the pro-inflammatory cytokine gene (TNF-α) in populations exposed to elevated PM2.5 and PM10 concentrations in Tashkent and Nukus compared to Samarkand. These results provide molecular evidence supporting the biological plausibility of PM-induced health effects in Central Asian populations and highlight the urgent need for targeted interventions.

Air Quality and PM Exposure in Uzbekistan

Our monitoring data revealed that Tashkent (PM2.5: 42.3 μg/m³, PM10: 74.8 μg/m³), Nukus (PM2.5: 38.5 μg/m³, PM10: 68.3 μg/m³), and Samarkand (PM2.5: 28.7 μg/m³, PM10: 53.2 μg/m³) all exhibited PM concentrations substantially exceeding WHO 2021 air quality guidelines. These values are comparable to levels reported in other severely polluted cities in developing countries, including several in South Asia and China. The PM2.5 levels in Tashkent (42.3 μg/m³) are notably similar to those in Almaty, Kazakhstan (population mean PM2.5: ~26.7 μg/m³), where a recent study found significant gene-environment interactions affecting asthma risk.

The consistent seasonal pattern observed, with winter peaks in all three cities, strongly implicates fossil fuel heating as a major source of particulate pollution, corroborating the findings of the UNEP report on Uzbekistan. The additional spring peaks in Nukus associated with dust storms originating from the Aral Sea region align with previous reports documenting the regional impact of desertification. The transboundary nature of these pollution events, with PM10 plumes reaching Nukus from hundreds of kilometers away, underscores the need for regional cooperation on air quality management, as emphasized by UNEP.

The PM2.5/PM10 ratios observed (0.54-0.57) suggest a balanced contribution of fine and coarse particles, with fine particles dominating in Tashkent due to vehicular and industrial emissions, while coarse particles contribute more substantially in Samarkand (ratio 0.54), potentially from construction activities and localized dust. The high proportion of days exceeding WHO guidelines (75-93% for PM2.5; 60-78% for PM10) indicates that most of the population in these cities is chronically exposed to unsafe air quality, a finding with serious public health implications.

GSTM1 and GSTT1 Expression: Antioxidant Defense Downregulation

Our finding of significant GSTM1 and GSTT1 downregulation in Tashkent and Nukus populations is biologically plausible and mechanistically consistent with the known toxicological effects of PM exposure. GSTM1 encodes an enzyme that catalyzes the conjugation of glutathione to reactive electrophiles and xenobiotics, facilitating their detoxification and excretion. Under conditions of oxidative stress induced by PM exposure, GSTM1 expression is expected to be upregulated as a compensatory response. However, chronic exposure to high levels of oxidative stress can overwhelm this response, leading to enzyme depletion, reduced mRNA stability, or epigenetic repression.

The more pronounced downregulation of GSTM1 compared to GSTT1 (2.1-fold vs. 1.6-fold in Tashkent) may reflect the fact that GSTM1 is more directly involved in the detoxification of PAHs, quinones, and other specific organic pollutants commonly found in urban PM from vehicular and industrial sources. The finding that the

downregulation was more severe in Tashkent (PM2.5 42.3 μg/m³) than in Nukus (PM2.5 38.5 μg/m³) suggests a possible dose-response relationship, which was further supported by the correlation and regression analyses.

These results are consistent with findings from the multi-omics systematic review, which demonstrated that PM exposure disrupts glutathione and antioxidant metabolic pathways. Studies included in that review showed that superoxide dismutase (SOD) and glutathione-related metabolites were frequently reduced following PM exposure, supporting the overall concept of redox imbalance. The inverse correlations between PM exposure and GSTM1/GSTT1 expression in our study (r=-0.61 to -0.58 for GSTM1; r=-0.43 to -0.41 for GSTT1) suggest a robust relationship that is likely clinically significant.

TNF-α Expression: Inflammatory Activation

The strong upregulation of TNF-α observed in Tashkent (3.4-fold) and Nukus (2.7-fold) populations is a critical finding that provides mechanistic insight into PM-induced systemic inflammation. TNF-α is a master regulator of the inflammatory response, mediating leukocyte recruitment, tissue injury, and the acute phase reaction. PM2.5 exposure has been shown to activate the NF-κB pathway, leading to increased TNF-α transcription. The consistent upregulation observed in the high-exposure cities and the robust positive correlation (r=0.72 between PM2.5 and TNF-α) strongly support the causal link between air pollution and inflammatory responses.

The higher TNF-α upregulation in Tashkent (3.4-fold) compared to Nukus (2.7-fold) may be related to the different chemical composition of PM in these cities. Tashkent's PM is dominated by traffic and industrial emissions, which contain higher levels of transition metals and PAHs that are potent inducers of oxidative stress and inflammation. Nukus, while experiencing high PM levels, may have a larger proportion of mineral dust (from Aral Sea and desert sources), which has been suggested to be less potent in inducing pro-inflammatory cytokine release compared to combustion-derived particles. However, dust PM can still induce significant inflammatory responses through the release of endotoxins and metal components.

The correlation coefficient between PM2.5 and TNF-α expression (r=0.72) indicates that over 50% of the variance in TNF-α expression is explained by PM2.5 exposure, controlling for other factors. This strong association suggests that TNF-α expression might serve as a useful molecular biomarker for air pollution exposure and its inflammatory effects, as highlighted by recent reviews on emerging biomarkers for PM-induced respiratory toxicity.

Age and Sex Differences in Susceptibility

The subgroup analysis revealing age-related differences in PM effects is consistent with the broader literature on vulnerability to air pollution. Older participants (>45 years) showed stronger associations between PM2.5 and both TNF-α upregulation and GSTM1 downregulation compared to younger adults. This may be due to age-related declines in antioxidant capacity, reduced DNA repair efficiency, and immunosenescence. Previous studies have also shown that elderly populations are more susceptible to the cardiovascular effects of air pollution, with proposed mechanisms including reduced vascular resilience and impaired compensatory responses.

The sex difference observed for TNF-α, with females showing stronger PM2.5 effects (β=0.61 vs. β=0.52 in males), aligns with findings from the study conducted in Tashkent showing that women exhibited greater susceptibility to AQI-related blood pressure increases . Biological mechanisms proposed for this sex difference include hormonal influences on inflammatory responses (estrogen may enhance immune reactivity) and differences in glutathione metabolism between sexes. The absence of a sex interaction for GSTM1 and GSTT1 suggests that detoxification enzyme regulation may be less sex-dependent than inflammatory responses, though this requires further investigation.

Implications for Public Health in Uzbekistan

The population-level genetic and molecular alterations observed in this study have significant public health implications. The downregulation of GSTM1 and GSTT1 suggests that populations in high-exposure cities may have reduced capacity to detoxify harmful pollutants, leading to increased oxidative damage and disease risk. The upregulation of TNF-α indicates a systemic inflammatory state that predisposes individuals to respiratory, cardiovascular, and metabolic diseases.

The fact that Tashkent (PM2.5: 42.3 μg/m³) and Nukus (PM2.5: 38.5 μg/m³) both exceed the WHO guideline (15 μg/m³) by more than 250% underscores the urgency of intervention. The UNEP report has already recommended several measures, including: (1) stepped-up air quality monitoring; (2) early warning systems; (3) targeted emission reduction plans for fossil fuel combustion; (4) promotion of cleaner mobility; and (5) regional cooperation on data sharing and forecasting.

Our findings add molecular evidence supporting these policy measures. The strong gene-exposure relationships observed suggest that reducing PM levels could directly mitigate the molecular damage, potentially reducing disease risk. The development of a precision public health approach, incorporating genetic susceptibility profiling alongside environmental monitoring, could help identify vulnerable subgroups and guide targeted interventions. This approach would be particularly valuable for the elderly and female populations identified in our subgroup analysis.

Strengths and Limitations

This study has several strengths. First, it is the first investigation of air pollution-gene expression relationships in Uzbekistan, addressing a critical knowledge gap in Central Asia. Second, the multi-city design allowed comparison of populations exposed to varying PM levels, enhancing causal inference. Third, the use of 12 months of continuous monitoring data provided robust exposure assessment. Fourth, the adjustment for key confounders (age, sex, BMI, smoking) strengthened the validity of the exposure-response relationships.

However, several limitations should be acknowledged. First, the cross-sectional design precludes establishing causality, though the robust exposure-response gradients observed support biological plausibility. Future longitudinal studies would be valuable to examine temporal relationships and cumulative effects of PM exposure on gene expression. Second, gene expression was measured only at a single time point, and diurnal variability cannot be assessed. Third, personal exposure to PM may differ from ambient monitor data due to time spent indoors, transportation choices, and activity patterns. While monitoring data provide a reasonable proxy for population-level exposure, individual-level exposure misclassification may occur. Fourth, the study did not include genetic polymorphism analysis (null genotypes of GSTM1/GSTT1) alongside expression analysis. Given the prevalence of null genotypes in many populations, future studies should examine genotype-expression interactions. Fifth, protein-level validation of gene expression differences was not performed, though the consistency of qRT-PCR findings with published literature supports their validity. Sixth, the study population was restricted to healthy, non-smoking adults, limiting generalizability to broader populations.

CONCLUSION

This study demonstrates that populations residing in Tashkent and Nukus, Uzbekistan, exposed to high levels of PM2.5 and PM10, exhibit significant alterations in the expression of oxidative stress and inflammation-related genes compared to the lower-exposure population of Samarkand. Specifically, GSTM1 and GSTT1 are downregulated, while TNF-α is strongly upregulated, with robust dose-response relationships observed. These molecular changes provide mechanistic insights into the health effects of air pollution in Central Asia and identify TNF-α as a potentially valuable biomarker for pollution-induced inflammation.

The findings highlight the urgent need for comprehensive air quality management strategies in Uzbekistan, including implementation of WHO-recommended measures to reduce PM emissions. Additionally, the identification of age- and sex-specific effects suggests that certain vulnerable subgroups require prioritized protection. Future research should extend these findings through longitudinal studies, incorporate genetic polymorphism analysis, and examine the clinical health outcomes associated with the observed gene expression alterations.

The integration of molecular biomarker science with environmental monitoring, as demonstrated in this study, offers a transformative approach for predictive, preventive, and personalized strategies to mitigate the health burden of air pollution in Uzbekistan and the wider Central Asian region.

Conflicts of Interest

The authors declare no conflicts of interest. No financial or personal relationships have influenced the design, conduct, analysis, or reporting of this research. The study was conducted independently without input from commercial entities that could affect its objectivity.

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