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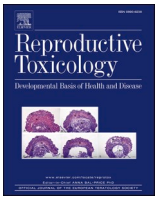

Urinary metabolite biomarkers of pregnancy complications associated with maternal exposure to particulate matter

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ABSTRACT

Particulate matter 2.5 (PM2.5) is associated with reproductive health and adverse pregnancy outcomes. However, studies evaluating biological markers of PM2.5 are lacking, and identifying biomarkers for estimating prenatal exposure to prevent pregnancy complications is essential. Therefore, we aimed to explore urine metabolites that are easy to measure as biomarkers of exposure.

In this matched case-control study based on the PM2.5 exposure, 30 high PM2.5 group (>15 μg/m^3) and 30 low PM2.5 group (<15 μg/m^3) were selected from air pollution on pregnancy outcome (APPO) cohort study. We used a time-weighted average model to estimate individual PM exposure, which used indoor PM2.5 and outdoor PM2.5 concentrations by atmospheric measurement network based on residential addresses. Clinical characteristics and urine samples were collected from participants during the second trimester of pregnancy. Urine metabolites were quantitatively measured using gas chromatography-mass spectrometry following multistep chemical derivatization. Statistical analyses were conducted using SPSS version 21 and MetaboAnalyst 5.0.

Small for gestational age and gestational diabetes (GDM) were significantly increased in the high PM2.5 group, respectively ($P = 0.042$, and 0.022). Fifteen metabolites showed significant differences between the two groups ($P < 0.05$). Subsequent pathway enrichment revealed that four pathways, including pentose and glucuronate interconversion with three pentose sugars (ribose, arabinose, and xylose; $P < 0.05$). The concentration of ribose increased preterm births (PTB) and GDM ($P = 0.044$ and 0.049, respectively), and the arabinose concentration showed a tendency to increase in PTB ($P = 0.044$).

Therefore, we identified urinary pentose metabolites as biomarkers of PM2.5 and confirmed the possibility of their relationship with pregnancy complications.

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1. Introduction

Particulate matter (PM) is a leading risk factor for cardiovascular and respiratory diseases and cancer [1]. PM2.5 (particles with an aerodynamic diameter of ≤2.5 μm) is absorbed through the human's respiration and has been reported to be associated with adverse pregnancy outcomes, including small for gestational age (SGA), preterm birth (PTB), gestational diabetes (GDM) and preeclampsia [2–8].

SGA (birth weight below the 10th percentile for gestational age), and PTB (delivery at <37 weeks of gestation) are complications of pregnancy that directly affect the prognosis of neonates [4–6,9–12] and GDM is also a risk factor for PTB and preeclampsia during pregnancy, and is associated with various complications during delivery in addition to fetal macrosomia and large for gestational age (LGA) [7,8,13]. Adverse pregnancy outcomes are caused by multifactorial risk factors, including environmental pollution; however, the pathogenesis of environmental exposure remains unclear [14,15].

Most existing studies on the relationship between various pregnancy complications and fine dust have been conducted by measuring outdoor air quality and not by measuring the indoor space where the subject spent most of the time [2,4,5,16]. As the majority of modern people spend 80–90% of their lives indoors, the importance of indoor air quality is gradually being emphasized [17,18]. Therefore, studies using indoor air quality measurements have the advantage of showing the relationship with diseases more clearly [17,19,20].

In addition, studies have been conducted to evaluate the mechanism of how exposure to PM affects fetal health, but there is a lack of biomarkers that can be easily bio-monitored [19,21–24]. Although there are studies on oxidative stress, inflammation, DNA damage, and epigenetic modulation as biomarkers of PM exposure, there are few biomarker studies in pregnant women [21].

Metabolomics is useful in understanding the mechanisms by which toxic substances impact the body, and subsequent metabolic responses to the toxicant [25]. Metabolomics, distinct from xenometabolomics which assesses the metabolism of exogenous pollutants, explores external factors influencing endogenous metabolites, providing novel insights into developing clinical biomarkers associated with pregnancy [26,27]. With the recent development of sensitive techniques, including gas or liquid chromatography-mass spectrometry or nuclear magnetic resonance, it has become a powerful tool for elucidating significantly altered metabolites and the subsequent understanding of biochemical processes involved in maternal or neonatal disease progression through pollutant exposure [28]. Urine metabolites are favored biofluids for analyzing the interactions of pregnant women with their environment. Clinical samples are easy to obtain, and the metabolites inside these samples clearly represent the complete spectrum of metabolic breakdown products in the maternal body. Previous studies have revealed that heavy metals significantly perturb urine metabolites, such as short-chain acids or amine metabolites [29]. A recent study has investigated the close relationship between environmental exposure to organophosphate pesticides or phthalates and urine metabolites [30]. Along with the advantages of urine metabolomics, the environmental effects on pregnant women have great potential for discovering prognostic and diagnostic biomarkers. [31].

Therefore, in this study, we aimed to examine whether the concentration of PM2.5 affects adverse pregnancy outcomes through actual indoor and outdoor particulate matter measurement. We also evaluated urine metabolite biomarkers of pregnancy-related complications caused by PM2.5 and indicators for biomonitoring PM2.5 exposure.

2. Materials and methods

2.1. Study design and collection of blood and urine samples

A matched case-control group based on the exposure [32] was selected by randomly selecting 30 high PM2.5 groups and 30 low PM2.5 groups depending on personal PM2.5, after matching maternal age and pre-pregnancy BMI from the Air Pollution on Pregnancy Outcome (APPO) study, an ongoing multicenter prospective cohort study to investigate the effects of particulate matter on mothers and fetuses between January 2021 and December 2023 in seven university hospitals in South Korea [33]. At the time of recruitment, the subjects were pregnant women before the second trimester of pregnancy without underlying diseases, and there were no pregnancy complications at the time of recruitment. According to the World Health Organization's (WHO) air quality guideline, a concentration of PM2.5 over 15 μg/m3 is considered as the High PM2.5 group, and below 15 μg/m3 as the Low PM2.5 group.

Basic demographic data and health-related characteristics, including age, pre-pregnancy BMI, socioeconomic status, and obstetric history, were collected and the presence or absence of complications including PTB, SGA, LGA, GDM, and preeclampsia at the time of delivery was collected. We collected 15 mL urine samples at regular outpatient visits during the second trimester of pregnancy. Urine samples were stored in conical tube and transferred to the institution (the Seegene Medical Foundation, Seoul, Korea) on the same day of collection by refrigerating to prevent deterioration (−80 ℃). The pregnancy outcomes were evaluated after delivery. (Fig. 1).

2.2. Particulate matter exposure assessment of subjects

Outdoor PM2.5 concentrations data were collected from a nearby urban atmospheric measurement network. The location of the nearby measurement station was obtained by collecting participants' residence information. Data from urban air monitoring stations were obtained from the Air Korea Database of the Korean Ministry of Environment (https://www.airkorea.or.kr/web). The indoor PM2.5, which was placed at breathing height in the living room of a subject's house, was measured using an AirGuard K (Kweather, Co., Korea) instrument. This device measures the sensor method and transmits the concentration information online every 1 min intervals. The measured indoor PM2.5 data were stored in an indoor air quality monitoring platform (IAQ Station) and recorded in real time using the Internet of Things (IoT) and Information and Communication Technology. We used a time-weighted average model to estimate individual PM exposure, which considers the duration and location of various activities to obtain more accurate measurements of individual PM exposure through time activity pattern analysis [34,35]. Indoor fine dust exposure was conducted for at least one week per quarter of pregnancy, and the calculation formula for estimating exposure was conducted according to the APPO study design [36]. According to the World Health Organization's (WHO) air quality guideline, a concentration of PM2.5 over 15 μg/m3 is considered as the High PM2.5 group, and below 15 μg/m3 as the Low PM2.5 group.

2.3. Targeted GC-MS metabolomics profiling

Urine metabolites were extracted using liquid-liquid extraction. Internal standard solution 100 μL (succinate-d_4, alanine-d_7 and tryptophan-d_5 10 μg/mL, in water) was spiked to 50 μL of urine. Urea-free urine was processed using a biphasic extraction-based protocol as previously described [37]. Methanol 150 μL were added at urea removed urine for polar metabolites extraction. Chloroform 150 μL were added to precipitate the protein and separate the layer. The processed samples were vortexed for 1 min and centrifuged at 13,000g for 5 min. The chloroform layer was transferred to another tube, and the same process was repeated. Separated polar layer was filtered with 0.45 μm PTFE (Polytetrafluoroethylene) syringe filter and evaporated with nitrogen gas. The processed samples were then dried overnight in a vacuum centrifuge. For methoxyamination, 50 μL of 20 mg/mL methoxyamine hydrochloride solution in pyridine was added and incubated at 37 ℃ for 90 min. Subsequently, 50 μL of N,O-Bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane solution was added for trimethylsilylation, and the sample was incubated at 75 ℃ for 1 h.

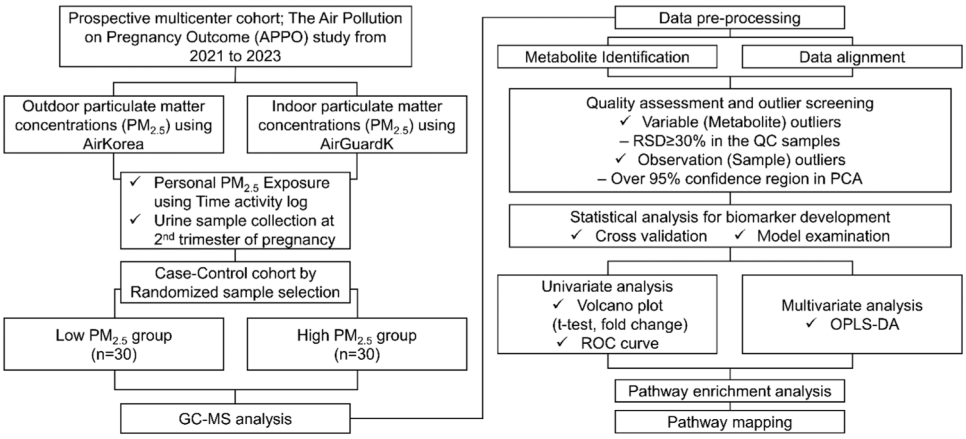


Fig. 1. Study flow chart. $PM_{2.5}$, particulate matter 2.5; GC-MS, gas chromatography-mass spectrometry; OPLS-DA, orthogonal partial least squares discriminant analysis; ROC, receiver operating characteristic.

Derivatized samples were centrifuged at 13,000 x *g* for 5 min and transferred to a glass vial. The derivatized urine metabolite extracts were analyzed using a Shimadzu QP2010 GC-MS system(Shimadzu Co.) equipped with a DB-5 ms column. One microliter of sample was injected in splitless mode and injection temperature was set at 270 ℃. The flow rate of the helium carrier gas was 1 mL/min. The GC oven initial temperature set was 70 ℃ (2 min of hold time), ramped to 100 ℃ at 4 ℃/min (held 3 min), ramped to 160 ℃ at 3 ℃/min (held 1 min), ramped to 200 ℃ at 4 ℃/min (held 2 min), and ramped 300 ℃ at 8 ℃/min (held 10 min) for a total run time of 68 min. Interface and ion source temperature was 300 ℃ and 250 ℃. Ionization was conducted in the electron impact (EI) mode, and MS data were acquired in full scan mode over the 40–600 *m/z* range.

Urinary metabolite profiles were analyzed using an optimized method to ensure reliability of the results. After excluding 10 metabolites with a relative standard deviation greater than 30% from the 24 QC samples, 45 metabolites (14 sugars, six sugar alcohols, seven sugar acids, 14 organic acids, and 4 amino metabolites) were used for multivariate statistical analysis (Supplementary Table 1. Metabolite identification).

2.4. Data processing and statistical analysis

Unsupervised principal component analysis (PCA) proved the reliability of the urine metabolite data. PCA reduces high dimensionality data into a single principal component (PC) by scoring each metabolite as an individual variable, followed by extraction of cumulative scores of variables as a single PC [38]. The data table of 45 metabolites (variables) and 84 samples (observations) (sample n = 60 and QC n = 24) pre-processed by Pareto scaling and sum normalization produced a PCA score plot with seven outliers from the confidence region. PCA was performed on 77 observations, including 27 high PM2.5, 26 low PM2.5, and 24 QC samples. The PCA score scatter plot with PC2 (12.6% variables) and PC3 (9.8% variables) clustered well between low and high PM2.5, while 24 QC samples were centered between the two groups, which indicates consistency in data acquisition and confidence in data processing and statistical analysis. (Supplementary figure 1. PCA score and scree plot) PC explains the variation of metabolites influenced by the maternal health response to PM2.5.

The clinical information of the case-control group was analyzed as follows: categorical variables were analyzed using the Chi-square test and, if the number of cells with an expected frequency less than 5 is more than 20%, Fisher's exact tests. Continuous variables were compared using Student's *t*-test, and if it does not follow a equal distribution, the Mann-Whitney *U* test or. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (version 20.0) (Chicago, IL, USA).

Metabolomic data were analyzed using Shimadzu GCMS Real Time analysis and postrun analysis. Metabolites were identified using the National Institute of Standards and Technology Mass Spectral Library (NIST08). The identification of sugar metabolites was confirmed by acquiring data from standard compounds, after which the retention indices and mass spectra were compared. All data were aligned using the MetAlign software, and parameters were set as described in a previous study [37]. The aligned peak lists were normalized to internal standard data. MetaboAnalyst 5.0 was used to acquire statistical analysis results. Following sum normalization and Pareto scaling, the processed data were used for multivariate statistical analysis. Principal component analysis (PCA) was used to assess data reproducibility and remove outliers. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and univariate statistical analyses, including Student's *t*-test, fold change (FC) analysis, and receiver operating characteristic (ROC) curves, were used to identify potential biomarkers. The elucidated biomarkers were used for pathway enrichment analysis and pathway mapping using VANTED software (version 2.8.3) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. OriginPro 2022 was used to produce a correlation heatmap between the metabolites and clinical parameters. Missing values were excluded by listwise deletion.

3. Results

3.1. Association between PM2.5 exposure and Pregnancy complication

The average exposure concentration of the High PM2.5 group was 22.76 μg/m^3, and Low PM2.5 group was 4.211 μg/m^3, showing a statistically significant difference ($P < 0.05$, Supplementary Table 2). There were no significant differences in clinical characteristics, including age, pre-pregnancy BMI, and socioeconomic status, between the High and Low PM2.5, but SGA and GDM were significantly increased in the high concentration group, respectively ($P = 0.042$, and 0.022, Table 1).

3.2. Urine metabolic responses to maternal PM2.5 exposure

Supervised statistical analysis using OPLS-DA and three different univariate statistical analyses (*t*-test, fold change, and receiver operating characteristic (ROC) curves) were used to elucidate urinary metabolite biomarkers. OPLS-DA distinctively clustered 27 high PM2.5 and 26 low PM2.5, based on 45 selected metabolite variables (Fig. 2a). OPLS-DA listed 15 biomarker candidates with variable importance for projection (VIP) values greater than 1.00 (Fig. 2b and Supplementary Table 3. VIP values). In unpaired *t*-tests and FC analysis, 17 metabolites satisfied a p-value <0.05, and FC (Log (High/Low) ratio) >1.5 or <0.66

Table 1
Clinical characteristics of study population.

Characteristics	Low $PM_{2.5}$ (n = 30)		High $PM_{2.5}$ (n = 30)		*P*-value
Age (years)	33.93	4.30	32.83	4.96	0.484[c]
Pre-BMI (kg/m^2)	21.93	3.54	21.78	2.94	0.352[c]
Education level					
High school graduation or below	2	6.67%	0	0%	0.143[a]
University graduates	28	93.33%	30	100%	
Monthly income					
<4 million won	9	36.0%	13	48.1%	0.73[a]
4–6 million won	7	28.0%	7	25.9%	
>6 million won	9	36.0%	7	25.9%	
Pregnancy methods					
Natural	25	83.3%	24	80%	0.587[a]
IUI	1	3.3%	0	0%	
IVF-ET	4	13.3%	6	20%	
Pregnancy outcome					
GAB (wks)	38.89	0.86	38.50	1.29	0.193[d]
PTB	0	0%	2	6.70%	0.157[b]
SGA	0	0%	4	13.30%	**0.042***[b]
LGA	0	0%	1	3.30%	0.321[b]
GDM	0	0%	5	16.70%	**0.022***[b]
Preeclampsia	0	0%	1	3.30%	0.321[b]
Anomaly	0	0%	1	3.30%	0.321[b]
NICU admission	0	0%	3	10%	0.08[b]
Neonate Sex					0.683[a]
Male	18	60%	16	53.30%	
Female	12	40%	14	46.70%	
Neonatal Outcome					
Birth weight (g)	3122.00	268.56	3184.33	493.14	0.595[c]
Birth height (cm)	49.55	1.78	49.54	2.01	0.866[c]
APGAR 1 min	8.70	0.95	8.10	0.96	0.171[d]
APGAR 5 min	9.63	0.67	9.17	0.87	0.238[d]

Categorical variables were expressed as frequencies (percentages) and analyzed using [a]Chi-square or [b]Fisher's exact tests (if the number of cells with an expected frequency less than 5 is more than 20%). Continuous variables were expressed as mean ± standard deviation (SD) and were compared using the [c]Student's *t*-test or [d]Mann–Whitney *U* test (if it does not follow a equal distribution),. BMI, body mass index; IUI, intrauterine insemination; IVF-ET, in vitro fertilization-embryo transfer; $PM_{2.5}$, particulate matter 2.5. GAB, gestational age at birth; APGAR, appearance, pulse, grimace, activity, and respiration; PTB, preterm birth; SGA, small for gestational age; NICU, neonatal intensive care unit. * $P < 0.05$, considered statistically significant.

(Supplementary figure 2. Volcano plot and Supplementary Table 4, 5 *t*-test, Fold change,). The area under curve (AUC) from ROC curve is generally used to distinguish differences between variables in metabolomics studies, and AUC above 0.7 is commonly considered to indicate that prognostic model is of fair quality [39,40]. Therefore, a criterion of an AUC above 0.7 was set for biomarker candidates, resulting in 23 metabolites meeting this condition in the comparison between low PM2.5 (n = 26) and high PM2.5 (n = 27) groups (Supplementary figure 3. ROC curves of 23 metabolites). Four sugars (arabinose, ribose, xylose, and ribofuranose), 2 sugar alcohols (xylitol and threitol), one sugar acid (ribonic acid), six organic acids (pyroglutamate, cis-aconitate, succinate, malonate, propanoate, and tartronic acid), and 2 amino acid metabolites (alanine and glucosamine) were confirmed as biomarkers. Among the metabolites, only the pentose metabolites, xylose (FC 5.15), ribose (FC 2.67), and arabinose (FC 2.58), were significantly upregulated at high PM2.5 (Fig. 2c), while the other biomarkers were downregulated (Fig. 2d).

3.3. Metabolic pathways associated with maternal and neonatal outcomes

For metabolic pathway enrichment analysis, MetaboAnalyst 5.0 measures the significance of associated pathways within whole metabolic pathway networks by calculating empirical p-value via mummichog algorithm [41]. Pathway enrichment analysis using the 15 metabolites identified 11 pathways that explained the influence of PM2.5 on metabolic pathway. Four pathways, pentose and glucuronate interconversions, citrate cycle, propanoate metabolism, and alanine, aspartate, and glutamate metabolism, showed p-values of < 0.05. (Fig. 3) Within the pentose and glucuronate interconversions, three upregulated metabolites (xylose, xylitol, and arabinose) were included, whereas the TCA cycle contained two metabolites (cis-aconitate and succinate). Propanoate metabolism consists of two metabolites (propanoate and succinate), and alanine, aspartate, and glutamate metabolism includes two metabolites (alanine and succinate).

The metabolic networks of the 11 pathways investigated the pathway alterations caused by maternal PM2.5 exposure (Fig. 4). The whole pathway map with upregulated or downregulated urinary metabolites centralized the pathway with the lowest p-value, pentose, and glucuronate interconversions, with close relationships with the other pathways. Three pentoses, which were the only upregulated metabolites, were closely associated with the potential upregulation of the pentose phosphate pathway and pentose and glucuronate interconversion. In particular, xylitol, a sugar alcohol, showed decreased expression in the high dose group, which may be related to an increase in xylose. Along with a consistent decrease in sugar alcohols, this could be a significant pathway for understanding the mechanism of PM toxicity in mothers. In the TCA cycle, metabolites, such as cis-aconitate and succinate, displayed decreased expression in the high PM exposure group. Propanoate metabolism; glutathione metabolism; and alanine, aspartate, and glutamate metabolism, which are associated with the TCA cycle, included significantly decreased metabolites, propanoate, pyroglutamic acid, and alanine, respectively.

3.4. Pentose concentration and adverse pregnancy outcome

According to the trend analysis for pregnancy complications according to the concentration group of pentose metabolites, it was confirmed that the increase in ribose was significantly related to PTB and GDM ($P = 0.044$ and 0.049, Table 2), and the arabinose concentration was significantly different from PTB ($P = 0.044$; Table 2).

4. Discussion

In this matched case-control study based on the PM2.5 exposure, we identified urinary pentose metabolites as biomarkers of PM2.5, and confirmed the possibility of their relationship with pregnancy complications.

Similar to the studies that observed the relationship between PM2.5, PTB, GDM, and SGA in previous studies, it was confirmed that GDM and SGA increased significantly in High PM2.5 group in this study. Although data were not provided, according to the results of the APPO study, PM2.5, increased the risk of PTB, GDM, and SGA in a prospective cohort study. Regarding the mechanism by which fine dust causes PTB, the mechanism caused by DNA damage through an increase in oxidative damage stress, especially mitochondrial DNA modification due to DNA methylation in the umbilical cord blood or placenta, and the mechanism causing changes in hormone concentrations as an endocrine disruptor have been studied [42,43]. In a study to identify the pathogenesis of gestational diabetes, fine dust absorbed into the lungs caused an increase in oxidative stress and systemic inflammation, and there was a mechanism by which particles were translocated into the circulation and increased endothelial dysfunction, increasing cardiovascular inflammation. In addition, each particle increases the stress of b-cells in the pancreas, causing dysfunction of b-cells, increasing inflammation of fat cells, resulting in changes in adipokines, and reducing muscle glucose uptake, increasing insulin resistance, an increase in oxidative stress through an increase in placental inflammation may also be a mechanism [7]. Despite these hypotheses, few studies have explored biomarkers to predict pregnancy complications associated with PM2.5. However, in this study, we found a metabolite associated with PM2.5 exposure and suggested its potential as a biomarker.

Among the increased metabolites, ribose is used as one of the

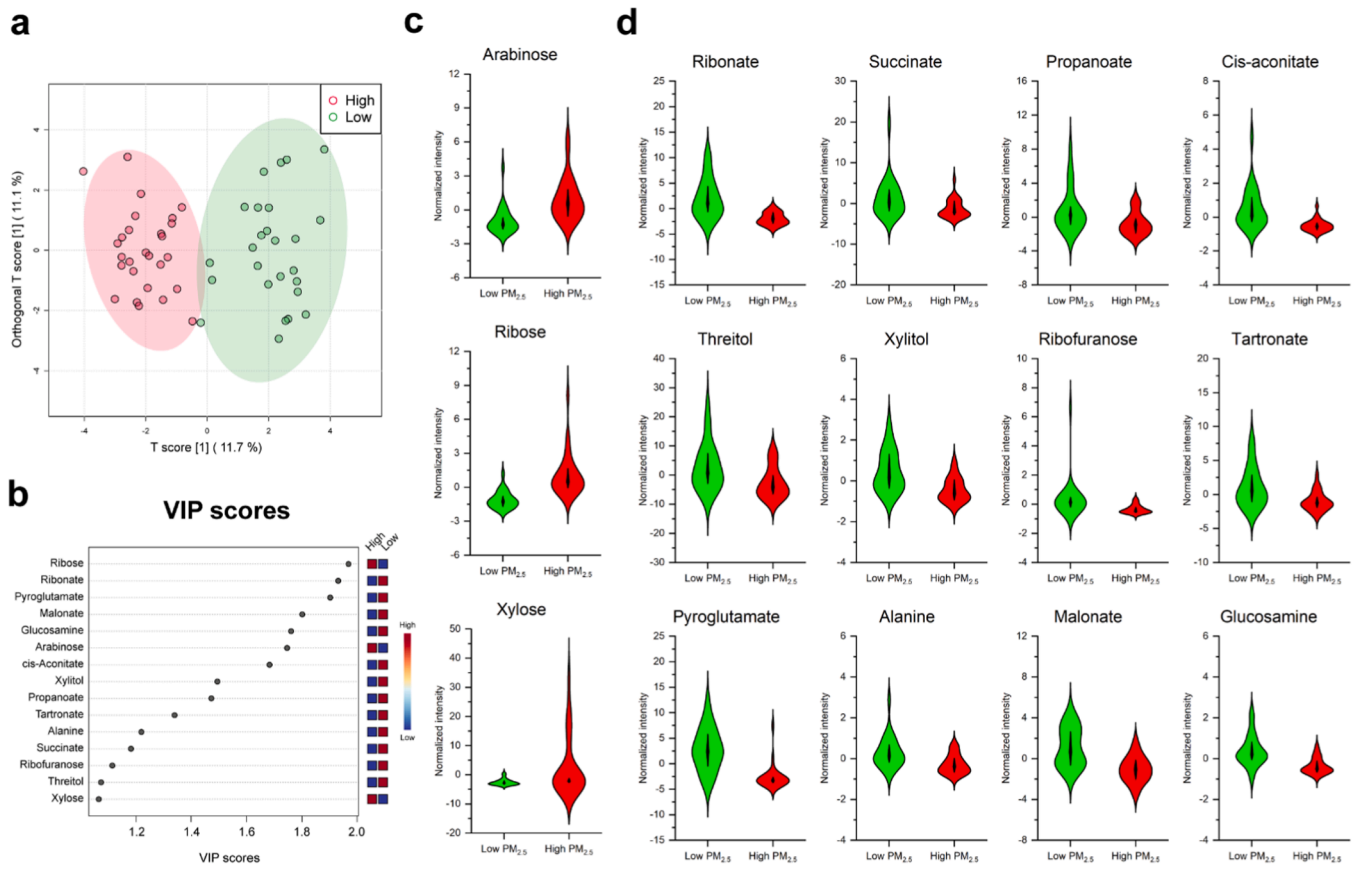
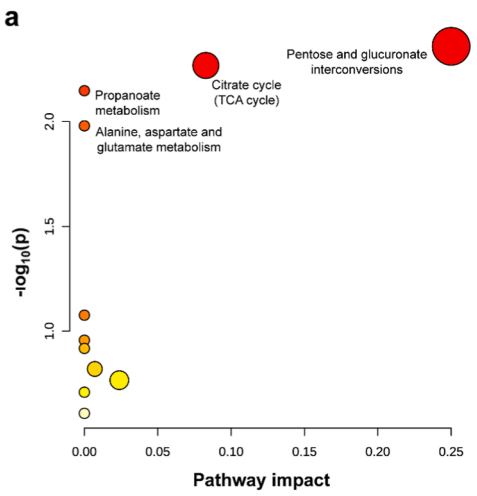


Fig. 2. Determination of PM2.5 exposure-related metabolites. (a) OPLS-DA score plot showing the separation of low PM2.5 (n = 26, green) and high PM2.5 (n = 27, red) groups. (b) Significant metabolites with a VIP score higher than 1.0. (c, d) Violin plots of 15 metabolites that are up-regulated (c) and down-regulated (d) in the PM2.5 exposure group. Green plots represent low PM2.5 levels and red plots represent high PM2.5 levels.



b

No	Pathway name	P value	Impact
1	Pentose and glucuronate interconversions	0.00437	0.25
2	Citrate cycle (TCA cycle)	0.00539	0.08276
3	Propanoate metabolism	0.00712	0
4	Alanine, aspartate and glutamate metabolism	0.01048	0
5	Butanoate metabolism	0.08401	0
6	Seleno compound metabolism	0.11058	0
7	Pentose phosphate pathway	0.12102	0
8	Glutathione metabolism	0.15168	0.00709
9	Glyoxylate and dicarboxylate metabolism	0.17159	0.02381
10	Amino sugar and nucleotide sugar metabolism	0.19589	0
11	Aminoacyl-tRNA biosynthesis	0.24713	0

Fig. 3. Metabolic pathway enrichment identified the most relevant metabolic pathways affected by PM2.5 exposure. (a) Pathway analysis plot with pathway impact (x-axis) and p-value (y-axis) represents the relevance of metabolic pathways. The size of the circle indicates the impact, while the color represents the significance, shown in (b) table. (b) Pathway lists associated with the urinary metabolites. Red highlight indicates the pathway with $p < 0.05$.

components of nucleic acids (DNA and RNA) to store and transmit genetic information, and plays an important role in several biological processes [44]. The cause of the ribose increase in urine through these functions may be disorders of ribose metabolism, tissue damage, or inflammation [45,46]. Previous studies have suggested that fine dust causes DNA damage through oxidative stress, and because one of the pathogeneses of PTB is oxidative damage and inflammation, it is assumed that an increase in ribose can be detected in this process.

According to a previous study on the increase of ribose in blood and urine when diabetes was present [47], there is a possibility that ribose may be an indicator of the occurrence of gestational diabetes. Additionally, poly (ADP-ribose) polymerase-1 (PARP1) expression has been reported as one of the mechanisms by which PM2.5 exhibits cytotoxicity and genotoxicity [48]. PARP1 regulates gene expression, and ribose is

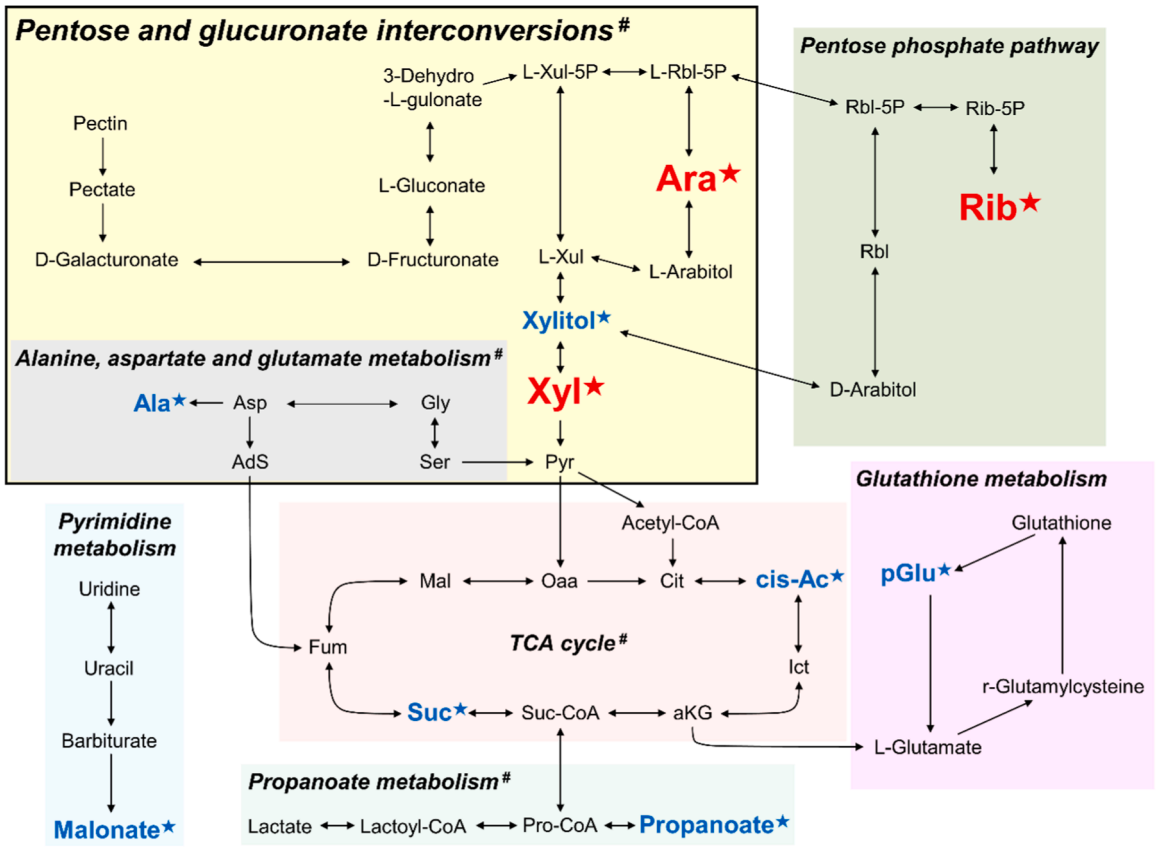


Fig. 4. Whole metabolic pathways of urinary metabolites associated with PM2.5 exposure. Each pathway statistically significant ($p < 0.05$)to the PM2.5 exposure was represented as # in the upper right corner. Metabolite biomarkers are colored red or blue (up or down-regulated in PM2.5 exposure) with a star in the upper right corner. Supplementary table 6 shows the abbreviation list of metabolites.

used in the process of repairing DNA damage; excess activity due to increased ribose may induce cell apoptosis [46]. Therefore, based on previous studies, this study suggests that increased ribose in urine is a biomarker for predicting PTB and GDM.

There are few studies on arabinose and xylose in humans, but they can increase in urine when there is a decrease or abnormality in the enzymes that degrade them [49]. Although this study did not show an association between increased xylose levels and GDM, previous studies have suggested that maternal urine xylose levels are a predictive factor for GDM [50]. Xylose and arabinose are related to PTB and bacterial vaginosis in the vaginal fluid, therefore, the increase in this metabolome may be related to its association with microorganisms [51,52]. Therefore, it is difficult to understand the pathogenesis of the arabinose and xylose excreted in this study, but they suggest the possibility of predicting factors for GDM and PTB, which may be related to the metabolism of microorganisms. Similar to previous studies showing that the microbiome influences the development of metabolic syndrome caused by environmentally harmful factors, this suggests that research may be needed to reveal its indirect effects [53].

The most important pathway through urine metabolites was the pentose and glucuronate interconversion pathway, which is known to play an important role in cell metabolism, according to the KEGG pathway database [54]. Several intermediate products of the pentose and glucuronate interconversion pathways are associated with glycolysis and the pentose phosphate pathway (PPP), which is involved in defense mechanisms against reactive oxygen species (ROS) [55]. Similar to previous studies, in which an increase in ribose in macrosomia cord blood and a related report on the pentose and glucuronate interconversion pathways were reported [56], the possibility that increased ribose in urine is related to macrosomia along with GDM can be suggested in this study. The citrate cycle plays a role in mitochondrial energy metabolism and plays an important role in almost all tissues and organs, especially in the energy-consuming tissues such as muscles, nerves, heart, and liver [54]. Propanate, alanine, aspartate, and glutamate metabolisms are also important pathways for energy metabolism [54]. Air pollutants are inhaled in the respiratory tract and deposited in the airway mucosa, causing an inflammatory response, chemical substances that increase oxidative stress through oxidation, and cellular damage [57]. It is thought to have an effect by acting as a toxic substance and causing cell damage.

Although the exact mechanism is difficult to understand, particulate matter is related to glucose metabolism through changes in the metabolomes of glucosamine, xylitol, tartraconic acid, succinic acid, ribofuranose, and thritol. Changes in alanine and pyoglutamic acid are related to the effects of amino acid metabolism. In addition, changes in propanoid acid levels suggest an association with lipid metabolism, whereas changes in malonic acid levels suggest an effect on organic compound synthesis. Therefore, PM can affect not only carbohydrate metabolism, but also amino acids, lipid metabolism, and organic compound synthesis.

To our knowledge, this is the first study to evaluate urine metabolites in pregnant Korean women with continuously measured indoor PM2.5 concentration. The strength of this study is that the case-control group was selected from a prospective multicenter cohort study that investigated the maternal and fetal health effects of PM on pregnancy in patients from various regions of South Korea. Compared with previous studies that measured only outdoor data, it was more reasonable to confirm the causal relationship between PM2.5 and pregnancy complications through the fine dust concentration measured using individual indoor air quality. In addition, through the discovery of biomarkers

Table 2
Pregnancy complication according to pentose concentration group.

Pregnancy complication	Ribose					Arabinose					Xylose				
	<25th % tile	25th-75th% tile	75th % tile<	*P* -value	*P for trend*	<25th % tile	25th-75th% tile	75th % tile<	*P* -value	*P for trend*	< 25th % tile	25th-75th% tile	75th % tile<	*P* -value	*P for trend*
Total complication	0 (0.0%)	12 (20.0%)	12 (40.0%)	**0.024***	**0.007***	2 (6.7%)	12 (20.0%)	10 (33.3%)	0.189	0.070	4 (13.3%)	7 (23.3%)	6 (20.0%)	0.732	0.651
PTB	0 (0.0%)	0 (0.0%)	4(13.30%)	**0.045** *	**0.044***	0 (0.0%)	0 (0.00%)	4 (13.3%)	**0.045***	**0.044***	0 (0.0%)	2 (3.3%)	2 (6.7%)	0.596	0.313
SGA	0 (0.0%)	6 (10.0%)	2 (6.7%)	0.448	0.468	2 (6.7%)	4 (6.7%)	2 (6.70%)	1.000	1.000	2 (6.7%)	4 (6.7%)	2 (6.7%)	1.000	1.000
LGA	0 (0.0%)	2 (3.3%)	0 (0.0%)	0.601	1.000	0 (0.0%)	2 (3.3%)	0 (0.0%)	0.601	1.000	0 (0.0%)	2 (3.3%)	0 (0.0%)	0.601	1.000
GDM	0 (0.0%)	4 (6.7%)	6 (20.0%)	0.126	**0.049***	2 (6.7%)	4 (6.7%)	4 (13.3%)	0.721	0.512	4 (13.3%)	4 (6.7%)	2 (6.7%)	0.721	0.512
Preeclampsia	0 (0.0%)	0 (0.0%)	2 (6.7%)	0.218	0.157	0 (0.0%)	0 (0.0%)	2 (6.7%)	0.218	0.157	0 (0.0%)	2 (3.3%)	0 (0.0%)	0.601	1.000
Anomaly	0 (0.0%)	0 (0.0%)	2 (6.7%)	0.218	0.157	0 (0.0%)	0 (0.0%)	2 (6.7%)	0.218	0.157	0 (0.0%)	2 (3.3%)	0 (0.0%)	0.601	1.000
NICU admission	0 (0.0%)	2 (3.3%)	4 (13.3%)	0.206	0.097	0 (0.0%)	2 (3.3%)	4 (13.3%)	0.206	0.097	0 (0.0%)	4 (6.7%)	2 (6.7%)	0.591	0.406

Categorical variables were expressed as percentages and analyzed using Chi-square or Fisher's exact tests and trend analyses. PTB, preterm birth; SGA, small for gestational age; LGA, large-for-gestational age; GDM, gestational diabetes mellitus; NICU, neonatal intensive care unit. $PM_{2.5}$, particulate matter 2.5. * $P < 0.05$, considered statistically significant.

capable of biomonitoring, we will be able to suggest that education regarding lifestyle changes, including smoking and cooking [17,20], and evaluate interventions such as using AMPK activators for metabolic disorders caused by PM2.5, may be considered before complications occur [58].

In our study, there are several limitations. While we conducted measurements of indoor PM2.5 for our subjects, the cumulative concentration during the entire pregnancy could not be calculated. Additionally, the indoor concentration values only represent those of the living room, introducing a potential limitation in capturing a comprehensive indoor exposure profile. Because the results of this study measured urine metabolites through excretion, there is a possibility that they may not show the same results as metabolites in the blood. In addition, it is difficult to determine the organ or cell involved in the mechanism of the study and it was difficult to analyze biological replicates with small sample volumes. However, many existing studies support the evidence that the composition of blood and urine metabolites is similar; therefore, mechanism analysis using urine metabolite results will be able to reflect the biological mechanism. Another limitation is that the exposure of the study subjects is based on the average exposure of entire pregnancy and does not reflect the exposure level at the time of sample collection, in addition, the analysis of complications was difficult to determine causality due to the limitation of a small number of subjects, making it difficult to confirm the causal relationship.

5. Conclusions

In this study, we identified urinary pentose metabolites (ribose and arabinose) as biomarkers of PM2.5 expose and confirmed the possibility of their relationship with pregnancy complications (PTB and GDM). Validation of the metabolite identified in this study as a marker for biomonitoring is required, and mediation analysis is needed to reveal the biological mechanism. As an exposure marker, this could be used as a tool to detect exposure to PM2.5, and prevent complications resulting from PM2.5.

Ethics approval and consent to participate

This study was approved by the Ethical Research Committee of Ewha Womans University Mokdong Hospital (EUMC 2021–04-032), Yonsei University Severance Hospital (4–2021-0414), Kangwon National University Hospital (KNUH-B-2021–04-012–008), Keimyung University Dongsan Medical Center (2021–04-073), Korea University Guro Hospital (2021GR0233), Ewha Womans University Seoul Hospital (2021–04-022), and Ulsan University Hospital (2022–04-020). All the participants provided written informed consent.

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CRediT authorship contribution statement

Lee Soo-Jeong: Resources. **Shim Minki:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Bae Jin-Gon:** Resources. **Park Mi Hye:** Resources. **Ko Hyejin:** Data curation. **Kim Young-Han:** Resources. **Na Sung Hun:** Resources. **Lee Dong-Kyu:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **You Young-Ah:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Lee Sun Hwa:** Methodology. **Lee Gain:** Methodology, Formal analysis, Data curation. **Hur Young Min:** Resources, Investigation, Conceptualization. **Kim Young Ju:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Kim Soo Min:** Formal analysis, Data curation. **Park Sunwha:** Writing – review & editing, Writing – original draft, Resources. **Cho Geum Joon:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Author Contributions

Sunwha Park enrolled the participants, interpreted the data, and wrote and edited the manuscript. Minki Shim obtained and processed the data, performed statistical analysis, and wrote the manuscript. Gain Lee curated the data and performed the experiments. Young-Ah You designed the study and edited the manuscript. Soo Min Kim performed the experiments. Young Min Hur developed the protocol and enrolled the subjects. Hyejin Ko performed the data curation. Mi Hye Park, Sung Hun Na, Young-Han Kim, Geum Joon Cho, Jin-Gon Bae, and Soo-Jeong Lee enrolled participants. Sun Hwa Lee developed the protocols and performed the experiments. Dong-Kyu Lee designed the experiments and prepared the manuscript. Young Ju Kim edited the manuscript, obtained the funding, and supervised the study. All the authors have read and agreed to the published version of this manuscript."

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2024.108550.

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