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Article

# Investigating the Dual Role of Trace and Heavy Metals in Pregnancy-Related Health Outcomes

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#### **Abstract**

This study investigates the levels of heavy metals (Cd, Pb, Cr, As) and trace elements (Zn, Cu, Fe) in pregnant women and examines their associations with adverse pregnancy-related clinical parameters. A cross-sectional study was conducted on 200 pregnant women at Thasala Hospital, Thailand, from January to July 2023. Blood samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and correlations were assessed using Spearman's rank-order correlation and coefficient tests. Iron showed the highest concentration among trace elements (751.78  $\pm$  120.45 mg/L), followed by zinc (11.89  $\pm$  2.12 mg/L) and copper (2.91  $\pm$  0.68 mg/L). Among heavy metals, lead had the highest concentration (0.06  $\pm$  0.02 mg/L), followed by chromium (0.03  $\pm$  0.01 mg/L), with arsenic and cadmium being lowest. Significant associations were identified between metal exposure and key clinical parameters including hematocrit, blood plasma glucose, and urine glucose levels (p < 0.05). The findings suggest that environmental exposure to both essential and toxic elements during pregnancy, even at low to moderate levels, may influence maternal health and highlight the need for preventive public health strategies within prenatal care programs.

Keywords: trace elements; heavy metals; ICP-OES; pregnancy health outcome

## 1. Introduction

Fetal growth and development are directly impacted by the nutritional state of the mother. Serum trace element levels are a crucial predictor of a mother's nutritional health throughout pregnancy. Trace elements are essential for pregnancy, and their deficiency can result in illnesses and physical anomalies that raise the risk of unfavorable pregnancy outcomes [1]. Among other elements, copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), and iron (Fe) are linked to unfavorable pregnancy outcomes. The previously mentioned trace element imbalance may have a direct correlation to several pregnancy issues, such as premature rupture of the membranes, fetal abnormalities, intrauterine growth restriction, stillbirth, miscarriage, and preterm delivery [2].



Heavy metals are persistent in the environment and can cause toxicity even at low levels; exposure to them has long been a matter for public health concern. Due to the worldwide increase in industrial pollution and man-made or natural combustion activities, we are all exposed to various environmental toxins, including heavy metals, organic hydrocarbons, and pesticides, either intentionally or unintentionally [3]. The atmosphere is full of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). These heavy metals are highly persistent in the environment and are both poisonous and bioaccumulative, making them a global hazard that endangers human development [4]. According to WHO statistics, all three of these heavy metals are included in the list of 10 substances that pose a risk to public health [5]. There are several ways that contaminated food, polluted groundwater, direct skin contact, and occupational exposure can expose human bodies to these hazardous metals. The accumulation of heavy metals in the body damages various types of cells and tissues. It is associated with organ malfunctions, particularly in the neurological, endocrine, and digestive systems, as well as kidney damage [6].

The placenta serves as a selective barrier during pregnancy, allowing nutrients and oxygen to reach the fetus while blocking the passage of harmful substances [7]. Transplacental transfer provides the fetus with heavy metals. Meanwhile, it has been demonstrated that the placenta is unable to prevent some teratogens from passing through. As a result, the placenta serves as the fetus's interface with the outside world and the mother, attaching it to the uterine wall and supplying it with blood from the mother [8]. Previously, using mouse embryonic stem cells or human induced pluripotent stem cells as an in vitro model for early embryos, it was revealed that several environmental contaminants produced aberrant DNA methylation at multiple gene loci at serum concentrations similar to those of maternal blood and cord blood [9,10]. Thus, in developing embryos, exposure to environmental toxins may lead to epigenetic malfunctions. The brain is one of the target organs for heavy metals, receiving special attention due to its widespread and particularly evident neurotoxicity, which can be observed even in the prenatal stage [11]. Cognitive impairments were further brought on by exposure to a heavy metal cocktail of Pb, Cd, and As, which led to substantial increases in intracellular calcium, oxidative stress, and cell death in the brain [12,13]. This suggests that exposure to heavy metals in humans begins early in life. In addition to cord blood, heavy metals might be detected in breast milk, which is the healthiest food for babies. The brain is one of the target organs of these heavy metals, and it develops quickly during the embryonic and infant periods [14]. Therefore, exposure to a heavy metal combination during pregnancy and breastfeeding may have detrimental effects. The risks to pregnancy outcomes and/or severe developmental impacts at levels below international limits appear to be a developing worry, although in utero exposure to heavy metals has been the subject of much research in recent decades. Therefore, there may be no thresholds for the effects of environmental exposures on pregnancy outcome or development, and the only sensible course of action is to limit environmental exposures for everyone.

Nonetheless, it is unclear how these trace components alter during pregnancy in Thai women due to racial, dietary, and environmental variables. It's also unclear how pregnant women's lack of trace minerals affects the outcome of their pregnancy. The purpose of this study is to measure the levels of trace elements (Zn, Cu, and Fe) and heavy metals (Cd, Pb, Cr, and As) in pregnant women and examine their associations with unfavorable pregnancy outcomes. This will enable the guidance of pregnant women regarding the intake of trace elements and the reduction of adverse pregnancy outcomes. Given the global concern on prenatal exposure to toxic metals and limited research from developing countries, this study address a critical gap in understanding environmental risk factors for adverse pregnancy outcomes in Thai women.

# 2. Materials and Methods

Study design, setting and sampling: A cross-sectional study was conducted at the Obstetrics and Gynecology Department of Thasala Hospital, Nakhon Si Thammarat, between January and July 2023. It included 200 pregnant women who attended antenatal care (ANC) at the Obstetrics and Gynecology Department of Tha sala Hospital, Nakhon Si Thammarat, during this period. The study

population were enrolled. Inclusion criteria: pregnant women aged over 18 years, no preexisting medical conditions or past pregnancy complications.

#### Clinical and Laboratory Variables

The validated questionnaires were used to gather data on mother occupation, education level, sociodemographic factors determinants (age, gestational age, and gestational body mass index, or G-BMI), and clinical parameters, including complete blood count (CBC), oral glucose tolerance testing (OGTT), blood pressure (SBP, DBP), obstetric history, including gravida (number of pregnancies; G), and urine analysis.

The venous maternal blood sample (3 mL) was collected at the time of visit in the ANC clinic. The blood samples were obtained by venipuncture and collected in ethylenediaminetetraacetic acid (EDTA) tubes. EDTA tubes as anticoagulants were used to prevent blood coagulation. The test tubes were placed in crushed ice and stored at  $-20^{\circ}$ C until required for analysis

# Determination of Metals in Maternal Blood

After being fully thawed at room temperature, the frozen blood samples were homogenized. 2 mL of concentrated nitric acid and 1 mL of 30% hydrogen superoxide were used to digest 1 mL of blood. The samples' solutions were then diluted with 15 mL of distilled water to detect metals [15]. Inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 730. Agilent Technologies, AU) was used to determine the levels of heavy metals (Cd, Pb, Cr, and As) and trace elements (Zn, Cu, and Fe). It was operated under the proper conditions, which included selecting the appropriate wavelength for each element (Table 1) with plasma argon flow rates of 12 L/min, auxiliary argon flow rates of 1.0 L/min, and nebulizer argon flow rates of 0.6 L/min, integration time of 100 s, and read delay of 15 s. Every chemical product employed was of the caliber of an analytical reagent. Solutions in 18.2 M $\Omega$  cm deionized water were produced. The standard XVI multi-element ICP standard solution from Merck KGaA, Darmstadt, Germany, was used to create calibration standards for every metal. Analysis did not include values below the limit of detection (LoD). The concentrations of elements are expressed as mg/L.

Elements	Spectral line (nm)	Plasma torch position	%RSE	$R^2$
Cu	327.395	Axial	8.78	.99964
Zn	213.857	Axial	8.00	.99964
Fe	238.204	Axial	8.45	.99972
As	193.696	Axial	10.93	.99993
Cd	214.439	Axial	1.62	.99999
Pb	220.353	Axial	1.57	.99995
Cr	267.716	Axial	1.23	.99999

Table 1. The analytical method parameters of ICP-OES.

#### Statistical Analysis

The data was analyzed using SPSS software version 28 (SPSS Inc., Chicago, IL, USA). Test results were displayed as Mean ± SD for sociodemographic traits, clinical parameters, heavy metals, and trace elements. The association between blood heavy metal and trace element concentrations and sociodemographic traits and clinical parameters was examined using the Spearman rank-order correlation matrix of heavy metals and trace minerals detected in plasma and correlation coefficients test. A p-value of less than 0.05 was deemed statistically significant.



# 3. Results

General Characteristics

200 pregnant women in all, and Table 2 lists all of their socio-demographic details. The pregnant women's ages ranged from 18 to 45 years old, with an average age of 30.2 years, a BMI of 27.5 kg/m2, and 40.5% of them in the third trimester. The majority had at least two pregnancies (44.5%) and had finished high school (52%). 67% were not housewives, and most had other employment. The average blood pressure was 115.2/74.7 mmHg, and the hematocrit was 35.8%. OGTT levels decreased throughout the 1-, 2-, and 3-hour averages of 158.5, 133.9, and 120.1 ng/dL, respectively, whereas fasting plasma glucose (FPG) averaged 85.4 mg/dL. The majority of women had negative urine protein and glucose tests. The majority were non-smokers (66%) and did not consume coffee (78%).

Table 2. Socio-demographic Characteristics of Pregnant Women.

	n (%) or mean ± SD						
Variables	Total (n=200)	1st trimester (n=71)	2nd trimester (n=45)	3rd trimester (n=81)			
Age (year)	$30.2 \pm 7.5$	$30.7 \pm 1.0$	30.2±1.2	29.8±0.7			
G-BMI (kg/m²)	$27.5 \pm 5.9$	$27.66 \pm 0.8$	25.77±0.8	28.32±0.6			
Education							
Less than high school	24 (12.0)	10 (14.1)	4 (8.9)	10 (11.9)			
High school	104 (52.0)	37 (52.1)	27 (60.0)	40 (47.6)			
Academic	72 (36.0)	24 (33.8)	14 (31.1)	34 (40.5)			
Gravidity							
1	60 (30.0)	21 (29.6)	12 (26.7)	27 (32.1)			
2	51 (25.5)	20 (28.2)	10 (22.2)	21 (25.0)			
≥3	89 (44.5)	30 (42.3)	23 (51.1)	36 (42.9)			
Maternal occupation							
Housewives	66 (33.0)	25 (35.2)	18 (40.0)	23 (27.4)			
Other	134 (67.0)	46 (64.8)	27 (60.0)	61 (72.6)			
SBP (mmHg)	$115.2 \pm 12.2$	$116.7 \pm 1.3$	$113.8 \pm 1.9$	$114.7 \pm 1.4$			
DBP (mmHg)	$74.7 \pm 9.9$	$77.0 \pm 0.9$	$73.8 \pm 1.3$	$73.3 \pm 1.3$			
Hematocrit (%)	0.		$35.5 \pm 0.5$	$35.6 \pm 0.4$			
Plasma glucose							
FPG (mg/dl)	$85.4 \pm 18.5$	$90.1 \pm 2.9$	$85.0 \pm 2.8$	$87.7 \pm 1.0$			
OGTT 1 hour	1505 - 771	175 1 . 11 0	1500	1400 + 24			
(mg/dL)	$158.5 \pm 67.1$	$175.1 \pm 11.8$	$150.2 \pm 6.9$	$148.8 \pm 3.4$			
OGTT 2 hour (mg/dL)	$133.9 \pm 39.9$	$140.0 \pm 5.3$	$133.2 \pm 7.3$	$129.2 \pm 3.1$			
OGTT 3 hour (mg/dL)	$120.1 \pm 33.8$	$117.9 \pm 4.5$	$121.9 \pm 6.7$	$120.8 \pm 2.4$			
Urine Protein							
Negative	184 (92.0)	62 (89.9)	43 (95.6)	77 (91.7)			
Positive	16 (8.0)	7 (10.1)	2 (4.4)	7 (8.3)			
Urine Glucose	, ,	, ,	, ,	, ,			
Negative			42 (93.3)	80 (95.2)			
Positive			3 (6.6) 4 (4.8)				
Smoke exposure	, ,	. ,	,	, ,			
Non-exposure	132 (66.0)	49 (69.0)	34 (75.6)	49 (58.3)			
Exposure	68 (44.0)	22 (31.0)	11 (24.4)	35 (41.7)			

P-BMI; Pregnancy BMI, SBP; Systolic blood pressure, DBP; Diastolic blood pressure, FPG; Fasting plasma glucose, OGTT; Oral glucose tolerance test, SD; standard deviation.

#### Trace Elements and Heavy Metal Concentrations

The mean concentrations of heavy metals and trace elements were shown in Table 3. According to the trace element levels group, the largest concentration is found in iron (Fe), followed by zinc (Zn) and copper (Cu), which have respective amounts of  $751.78 \pm 120.45$  mg/L,  $1187.20 \pm 211.38$  µg/dL, and  $294.72 \pm 67.19$  µg/dL. Among all the elements examined, arsenic (As) and cadmium (Cd) had the lowest concentrations, while lead (Pb) had the highest concentration, averaging  $5.59 \pm 1.61$  µg/dL. Chromium (Cr) came in second with an average of  $2.80 \pm 1.47$  µg/dL.

**Table 3.** Trace elements and heavy metal concentrations ( $\mu$ g/dL).

Elements	Mean	SD	Range
Trace elements			
Cu	294.72	67.19	145.00 - 525.00
Zn	1187.20	211.38	479.00 - 1971.00
Fe*	751.78	120.45	417.13 - 1018.87
Heavy metal			
As	1.87	1.43	ND - 6.00
Cd	0.98	0.27	ND - 4.00
Pb	5.59	1.61	2.00 - 11.00
Cr	2.80	1.47	1.00 - 16.00

SD; standard deviation, ND; not detected, \*mg/L.

The relationships between Cu, Zn, Fe, As, Cd, Cr, and Pb in blood are displayed in Table 4. The major heavy metals in blood have substantial Spearman rank correlation values (p < 0.05).

Table 4. Correlations between heavy metal concentrations (Spearman's rho).

	Cu	Zn	Fe	As	Cd	Cr	Pb
Cu	1.000						
Zn	.286**	1.000					
Fe	.258**	.485**	1.000				
As	159*	074	151*	1.000			
Cd	159*	074	151*	1.000**	1.000		
Cr	.047	.051	.047	.094	.094	1.000	
Pb	.110	.244**	.198**	.200**	.200**	.168*	1.000

<sup>\*\*.</sup> Correlation is significant at the 0.01 level (2-tailed), \*. Correlation is significant at the 0.05 level (2-tailed).

Correlation Coefficients of Selected Heavy Metals and Biological Parameters

Table 5 displays the correlations between main heavy metal levels and socio-demographic traits. We discovered modest but statistically significant connections between biological indicators and heavy metal levels based on the Spearman rank correlation values. The hematocrit ( $r_s$  = 0.174, p < 0.05) and coffee drink ( $r_s$  = -0.188, p < 0.01) were significantly correlated with the blood concentration of the heavy metal Cu; the hematocrit ( $r_s$  = -0.188, p < 0.01), OGTT 2 hour ( $r_s$  = -0.174, p < 0.05), and OGTT 3 hour ( $r_s$  = -0.220, p < 0.01) were significantly correlated with the level of Zn. As and Cd levels were correlated with urine glucose ( $r_s$  = -0.184, p < 0.01); the level of Cr was correlated with FBS ( $r_s$  = -0.141, p < 0.05); the level of Pb was significantly associated with the hematocrit ( $r_s$  = -0.219, p < 0.01); and the level of Fe was significantly associated with the hematocrit ( $r_s$  = -0.188, p < 0.01) and OGTT 2 hour ( $r_s$  = -0.162, p < 0.05). All of these relationships were statistically significant.

Table 5. Correlation Between Trace Element and Heavy Metal Levels with Health Effects in Pregnant Women.

Variables -			Correla	tion Coeffi	cient		
variables	Cu	Zn	Fe	As	Cd	Cr	Pb
Pregnancy-BMI	.023	.028	.051	.049	.049	.051	.121
SBP	074	.026	.097	.037	.037	.003	.044
DBP	099	.022	.122	.067	.067	024	.085
Hematocrit	174*	.188**	.274**	.093	.093	031	.219**
Urine protein	079	084	003	.032	.032	049	006
Urine glucose	126	036	.064	184**	184**	126	016
FBS	162*	030	.054	.043	.043	141*	.046
OGTT 1 hour	038	.119	.095	.032	.032	.005	060
OGTT 2 hour	026	.174*	.162*	.023	.023	.121	016
OGTT 3 hour	.101	.220**	.110	.006	.006	.072	053

<sup>\*\*</sup>Correlation is significant at the 0.01 level (2-tailed), \*Correlation is significant at the 0.05 level (2-tailed).

#### 4. Discussion

The levels of heavy metals (Cd, Pb, Cr, and As) and trace elements (TE) (Zn, Cu, and Fe) in pregnant women will be evaluated, and their correlation with unfavorable pregnancy outcomes will be examined. Numerous illnesses may be linked to deviations in the normal metabolism of vital components. Certain trace elements are related to the manufacture of hormones, while others are components of enzymes. Disease can result from both pathologically high and abnormally low quantities of some substances in the body [16]. The most often used test material as a biomarker to identify excess or deficiency of elements, particularly hazardous metals, is blood. Numerous elements are reflected in blood; however, their levels frequently vary across a broad range of quantities [17]. It is often challenging to identify a range of reference concentration levels due to considerable individual variability. However, little is known about the factors underlying worse pregnancy outcomes. In recent years, several studies have proposed a link between exposure to heavy metals, including Pb and Cr, and unfavorable pregnancy outcomes [18,19].

Although there is evidence that the levels of some heavy metals and trace minerals in our research sample are comparable to those in pregnant women in the United States in general [20], comparisons must consider certain measurement limitations. We measured the recent exposure levels of heavy metals and TE using whole blood in an EDTA tube. It is unclear how precisely and stably plasma reflects the actual exposure levels of these chemicals in the human body throughout pregnancy, despite its use in earlier research to quantify concentrations of these substances. Moreover, directly comparing chemical concentrations between populations is challenging. Earlier research has measured chemicals in various biomarkers (e.g., whole blood, urine, nails), at multiple stages of pregnancy (e.g., first, second, and third trimester), and/or with different laboratory equipment, all of which may have an impact on the reported chemical values [21]. Based on these findings, we investigated the relationships between maternal outcomes and exposure to a combination of heavy metals and trace minerals during pregnancy. We found that the combination of all compounds showed a positive correlation with blood sugar, urine glucose, and baseline hematocrit in cross-sectional studies. After separating this mixture, we found that Zn, Fe, and Cr were significantly and favorably correlated with blood sugar levels. At the same time, Cu, Zn, Fe, and Pb were significantly and favorably correlated with hematocrit. As and Cd were only significantly and favorably correlated with urine glucose.

Our research on maternal outcomes and vital trace minerals, including Cu, Zn, and Fe, contributes to a growing body of knowledge on the negative health implications of these substances. Drinking water, such as that initially drawn in the morning after spending the night in copper pipes and brass faucets, is the primary source of copper exposure. Most of the Fe and Zn that people consume each day comes from their diet, which includes green vegetables, dairy products, and seafood [20]. During pregnancy, these substances exhibited a slight positive correlation with blood

sugar and hematocrit levels. Microelements Cu, Zn, and Fe are essential for living organisms to function correctly. These components affect enzyme function, control gene expression, contribute to protein synthesis, and are involved in various activities, including cellular metabolism and antioxidant and anti-inflammatory defenses [22]. The development of the fetus, the health of the infant, and the health of expectant mothers are all significantly impacted by Fe, Cu, and Zn. Pregnancy issues, including anemia, induced hypertension, low birth weight, preeclampsia, and postnatal difficulties, are less likely to occur when these components are present in women's bodies at the right concentration [23].

It is well recognized that several heavy metals, including As, Cd, Hg, and Pb, produce free radicals, which can cause oxidative stress and damage to cells. Prenatal oxidative stress impairs fetal growth by interfering with placental formation, function, and remodeling. The erythrocyte, which may be a suitable biological matrix for some metals but not all, was used in this investigation to test the metals. Erythrocyte Cd, for example, may indicate both recent and cumulative exposure [24]. Blood As might not be a promising biomarker for cumulative exposure, in contrast to toenail or hair As. As in blood stands for total As, which is quickly eliminated in the urine (between 50% and 90% in 2-4 days). The inability of blood to distinguish between less hazardous organic forms and more lethal inorganic forms is another drawback [25]. However, when the exposure source is constant, blood is a valuable biomarker for chronic exposure and has a significant correlation with urine. Our data also showed a correlation between urine glucose and As and Cd levels, which is consistent with our findings.

We discovered a statistically significant correlation between hematocrit and blood lead levels in our investigation. According to a previous study, anemic pregnant women had considerably higher blood lead levels, and there was a negative correlation between lead levels and hemoglobin concentration. Increased bone remodeling during pregnancy is linked to the endogenous release of stored lead from bones, which accounts for 95% of the body's total Pb burden, into the bloodstream [26]. Negative consequences for both mothers and newborns have also been linked to elevated lead levels. Pregnant women should have their serum blood lead levels checked in a primary care environment, particularly if they are anemic or do not respond to standard iron therapy. To promote the health of mothers and newborns, more awareness must be raised about the need to prevent lead intake among pregnant women through limited cosmetic usage, vermillion use, PICA, and occupational exposure to lead. Women who live in locations with lead-based businesses or untreated water sources must have blood lead levels measured as part of a mandatory prenatal examination. Furthermore, it has been shown that higher lead absorption occurs when there is an inadequate supply of calcium, zinc, and iron. Lead has a broad range of toxicity to biological macromolecules, interfering with the stability of subcellular structures by displacing specific physiological metal cofactors from active sites and disrupting normal enzymatic functions. Pb-toxicity-related anemia is one of the most traditional signs that are frequently seen following a prolonged exposure to lead [27].

The study has some limitations. Although the participants represented all ages and gestational periods, the relatively small sample size precluded a comprehensive assessment of trace element levels in the pregnant populations at Thasala Hospital. Future research should confirm our results with a larger cohort and more robust experimental design. Additionally, further studies should evaluate other potential influencing factors such as parity, lifestyle, nutritional supplement intake, and genetic variations, all of which may affect the metabolism and accumulation of trace elements and heavy metals in pregnant women. Despite these limitations, our findings are consistent with international research. For example, studies conducted in China have shown that simultaneous exposure to arsenic, lead, thallium, and nickel during early pregnancy increases the risk of gestational diabetes mellitus, with arsenic playing a major role. This association is thought to result from arsenic's disruption of glucose metabolism and delays in insulin secretion following glucose intake in pregnant women [28]. These studies highlight the global relevance of our findings and underscore the importance of addressing environmental metal exposure in prenatal health strategies.

Given the limited available data from Southeast Asia, particularly Thailand, our study importantly fills a critical knowledge gap and highlights the urgent need for environmental monitoring programs and targeted public health interventions to reduce exposure risks among pregnant women. This would facilitate the implementation of preventive measures, ultimately protect maternal and fetal health, and align with global health priorities.

# 5. Conclusions

We demonstrated novel and significant association between maternal health status throughout the gestational period and exposure to heavy metals and trace minerals among pregnant women at Thasala Hospital. We found statistically significant correlation between exposure to heavy metals and trace minerals and key clinical indicators such as hematocrit, blood plasma glucose, and urine glucose levels. Notably, the highest exposure observed was to lead, highlighting that despite current efforts to mitigate environmental pollution, lead exposure remains a substantial public health concern, particularly for pregnant women and their offspring, who constitute vulnerable populations. These results highlight the urgent need for targeted interventions to minimize environmental metal exposure among pregnant women. We strongly recommend routine screening of blood lead levels during the preconception and early prenatal periods for high-risk women based on their personal, social, professional, and environmental factors. Public health education programs should be strengthened to increase awareness about the harmful effects of lead exposure, and regulatory measures must be enforced by the government to limit environmental exposure to lead effectively. These measures would significantly contribute to improved maternal and neonatal health outcomes, aligning closely with global public health objectives.

**Author Contributions:** Conceptualization, T.S., T.K., and S.Y.; methodology, validation and analysis, T.S., D.W., W.H. and S.Y.; writing-original draft preparation, T.S., D.W., and K.S.; writing-review and editing, T.S., T.K., and S.Y.; supervision, T.K., and S.Y. All authors have read and agreed to the published version of the manuscript..

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**Institutional Review Board Statement:** The objectives, procedure, and voluntary nature of the study were clearly explained to the participants and provided written informed consent before data collection. All methods in the present study were conducted following the Helsinki Declaration for research involving human subjects. This research has been approved by the Ethics Committee in Human Research at Walailak University (WUEC-23-151-01).

Informed Consent Statement: All subjects provided written informed consent.

**Data Availability Statement:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

#### **Abbreviations**

Copper
Zinc
Calcium
Magnesium
Iron
Lead
Cadmium



As Arsenic Hg Mercury

G-BMI gestational body mass index
CBC Complete blood count
FPG fasting plasma glucose
OGTT Oral glucose tolerance testing
SBP Systolic blood pressure
DBP Diastolic blood pressure

G Gravida

ICP-OES Inductively coupled plasma optical emission spectroscopy

LoD Limit of detection

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