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Maternal hair lead and cytokine pro-inflammatory effects in preterm birth

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ABSTRACT

This case-control study analyzed the lead (Pb), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) levels in pregnant women with preterm birth (PTB) in Central Java, Indonesia. Hair samples from 72 pregnant women were collected non-invasively. The prenatal exposure to Pb was determined with the total reflection X-ray fluorescence (TXRF) method. Serum IL-6 and TNF-α were examined using enzyme-linked immunosorbent assays (ELISA). The Pb concentration in hair was slightly higher in women with PTB than those without PTB; however, this difference was not statistically significant. An elevated hair Pb level was not associated with increased PTB risk (OR 24.69, 95% CI 0.93–653.82, p>0.05). A serum TNF-α level ≥27 pg/ml, a serum IL-6 level ≥9 pg/ml, and the spouse's smoking frequency were significantly associated with increased PTB risk (TNF-α OR 42.25, 95% CI 5.26–339.61; IL-6 OR 22.33, 95% CI 3.12–158.54; spouse's smoking frequency OR 1.28, 95% CI 1.09–1.5), while the maternal hemoglobin concentration significantly decreased PTB risk (OR 0.43, 95% CI 0.2–0.927). This study demonstrates that maternal hair Pb concentration has no significant relationship with PTB. Serum TNF-α, IL-6, and the spouse's smoking frequency potentially increased PTB risk, while the maternal hemoglobin level is a protective factor.

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1. INTRODUCTION

Lead (Pb) is a toxic metal used in industrial work as a battery, paint, cable coating, welding material, bullet ammunition, and solder. Human activities spread Pb pollution in the air, water, and soil [1], [2]. Pb enters the body by ingestion and inhalation. It is excreted in the feces, urine, sweat, saliva, hair, nails, and breast milk [3]. Numerous toxic effects of Pb have been reported, including miscarriage, stillbirth, preterm birth (PTB), low birth weight, and other fetomaternal outcomes. Moreover, children are more vulnerable to Pb exposure: This group shows 4–5 times higher Pb absorption than adults and they are also exposed to lead in-utero, resulting in adverse neurobehavioral developmental effects [4]. Because Pb pollution is still a concerning problem, Pb use has been prohibited.

PTB contributes to two-thirds of perinatal mortality. It has multiple factors, including inflammation [5]–[7]. An in vivo study showed that Pb exposure could alter inflammation by increasing the release of proinflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [8]. Cytokines are pyrogenic molecules that induce the synthesis of prostaglandin, which is essential in the delivery process [9], [10]. Labor induction before 37 weeks of gestational age could trigger PTB [11]. Based on existing theoretical findings, we wanted to know whether maternal Pb levels and maternal serum cytokine levels are interrelated and affect PTB.

In previous studies, there have been variable results regarding the effect of Pb exposure on PTB [12]–[16]. Most of these studies used invasive methods to examine heavy metal levels, but mothers and their infants require a non-invasive method to avoid inducing pain during the sampling procedures. Hair sampling is painless, and total X-ray fluorescence (TXRF) can measure hair elements, including Pb, with high sensitivity and low limit of detection [17], [18]. This study presents a non-invasive method to assess the association between PTB and Pb exposure and proinflammatory cytokines.

2. METHOD

2.1. Research subject

This study was conducted from May to December 2022. It involved 72 mothers who underwent labor in three cities in Central Java Indonesia: Semarang, Salatiga, and Jepara. The case group comprises mothers with PTB, while the control group comprises mothers with term labor. The inclusion criteria were mothers aged 20–35 years in labor, a resident of Central Java during pregnancy, a menstrual cycle of 24–38 days, and a body mass index (BMI) of 18.5–25. The exclusion criteria included twin pregnancy, a history of diabetes mellitus, chronic hypertension, hormonal contraception usage within three months before pregnancy, infertility, pregnant with the help of *in vitro* fertilization (IVF), stillbirth or fetal abnormality, history of a pathological condition of the uterus and previous PTB, polyhydramnios, a history of infection during pregnancy (HIV, hepatitis B, and syphilis), and inability to collect a hair sample.

2.2. Measurement of Pb in maternal hair

Hair samples were cut 3 cm from the scalp with sterilized scissors and kept in a sterile clip plastic. The collected hair sample was washed by soaking in acetone solution and put in a Bransonic Ultrasonic Cleaner within 5 minutes. After being incubated for around 30 minutes, the samples were dried at room temperature for eight hours. The samples were cut into 5-mm-long segments and measured using Teflon glass. The samples underwent digestion by adding a 5 ml mixture of HNO_3/H_2O_2 (3:1, v/v). Then, 10 µl of gallium, 5 µl of palladium, and 100 µl of polyvinyl alcohol (PVA) were added to the sample. The mixture was boiled at 110°C for 30 minutes. After that, 20 µl of the sample was dropped onto a total reflection X-ray fluorescence (TXRF) quartz disk. The disk was dried on a hot plate at 80°C and then examined with TXRF to determine the Pb content. The tools, materials, and work areas for hair sample preparation were made of plastic, Teflon, or silicon, and were free from contamination with other metals. The procedure was based on ISO/TS 18507:2015.

2.3. Measurement of IL-6 and TNF-α in maternal serum

Venous blood was taken within 48 hours after delivery in an EDTA tube. The sample was centrifuged at 3,000 rpm for 15 minutes. The serum was removed and stored in a freezer at -20°C until analysis. IL-6 and TNF-α levels in maternal serum were measured with enzyme-linked immunosorbent assay (ELISA) method.

2.4. Data analysis

SPSS Statistics version 21 was used for statistical analysis. The Kolmogorov–Smirnov test was used to determine whether the data followed a normal distribution. Subsequently, correlation analysis was performed. A bivariate comparative test was performed, followed by multivariate analysis for Hosmer–Lemeshow-compliant variables.

2.5. Ethical consideration

The study was performed by following the Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from all participants. The Health Research Ethics Committee, Faculty of Medicine Universitas Diponegoro, provided ethical clearance (study reference number 125/EC/KEPK/FK-UNDIP/V/2022).

3. RESULTS AND DISCUSSION

3.1. Characteristics of the Study Subject

Table 1 shows the subject characteristics, including possible Pb exposure and birth outcome. There were no significant differences in maternal parity, education, occupation, income, age, height, weight, body mass index (BMI), upper arm circumference, and hemoglobin level. In the case group, the median gestational age was 33.5 (25–36) weeks, and 35.7% of the subjects had premature membrane rupture (PROM). In the case group, 57.1% of the infants were male and the mean ± standard deviation birth weight was 1887.68±497.8 g.

Table 1. Subject's characteristics

Variables		N (%) or mean ± standard deviation; median (min–max)		p-value
		Case (n=28)	Control (n=44)	
Parity	Nullipara	12 (42.9%)	10 (22.7%)	0.071[a]
	Multipara	16 (57.1%)	34 (77.3 %)	
Education	<12 years old	9 (32.1%)	10 (22.7%)	0.377[a]
	≥12 years old	19 (67.9%)	34 (77.3%)	
Occupation	Employee	11 (39.3%)	22 (50%)	0.374[a]
	Housewife	17 (60.7%)	22 (50%)	
Income	<DMW	22 (78.6%)	27 (61.4%)	0.127[a]
	≥DMW	6 (21.4%)	17 (38.6%S)	
Maternal age		27±5.08; 26.5 (20–35)	28.05±4.79; 27.5 (20–40)	0.381[c]
Height (cm)		155.36±5.40; 155 (146–167)	155.22±5.19; 155 (136–168)	0.880[d]
Weight (kg)		53.4±6.88; 54 (40–66)	52.71±6.98; 52 (41–68)	0.680[c]
Body mass index (kg/m^2)		22.12±2.59; 22.13 (17.54–26.67)	21.88±2.69; 22.69 (17.29–27.06)	0.903[d]
Upper arm circumference (cm)		26.61±3.41; 26 (21–35)	25.6±2.54; 25.5 (20–35)	0.292[d]
Gestational age (weeks)		32.21±3.32; 33.5 (25–36)	38.95±1.2; 39 (37–42	0.00 [d]*
Hemoglobin		11.45±1.13; 11.5 (9–13.1)	11.82±1.21; 11.85 (7.1–14.9)	0.218[d]
Premature membrane rupture	Yes	10 (35.7%)	0	0.00[b]*
	No	18 (64.3%)	44 (100%)	
	No	19 (67.9%)	24 (54.5%)	
History of house renovation in the past 10 years	Yes	11 (39.3%)	27 (61.4%)	0.067[a]
	No	17 (60.7%)	17 (38.6%)	
Living in a high-traffic area[s]	Yes	8 (28.6%)	7 (15.9%)	0.161[a]
	No	20 (71.4%)	37 (84.1%)	
Family history of cigarette smoking	Yes	23 (82.1%)	32 (72.7%)	0.266[a]
	No	5 (17.9%)	12 (27.3%)	
Spouse's smoking frequency (cigarettes/day)		8.04±6.57; 6 (0–24)	5.4±4.84; 6 (0–18)	0.110[c]
Infant's sex	Female	12 (42.9%)	17 (38.6%)	0.722[a]
	Male	16 (57.1%)	27 (61.4%)	
Infant's birth weight (g)		1887.68±497.8; 1850 (900–3000)	3110.25±398.36; 3100 (2500–4400)	0.00[c]*

Note: [a]=Chi-square test; [b]=Fisher's exact test; [c]=independent samples T-test; [d]=Mann–Whitney test; [e]=based on PERDA No 3 of 2014 concerning 12 years of compulsory education; [f]=based on the district minimum wage (DMW) of Central Java (Governor Decree Number 561/54 of 2022 set the minimum wage at IDR 2,272,025); [g]=traffic area is based on a 5-point Likert scale completed by the subject (a score of 4–5 indicates a high-traffic area); *p<0.05

3.2. Analysis of Pb levels in hair from mothers with PTB

The median Pb maternal hair level in all subjects was 1.5 (1.1–2) ppm, see Table 2. The case group had a slightly higher median Pb hair level than the control group, 1.5 (1.12–2) versus 1.4 (1.1–1.9) ppm, but the difference was not significant, see Figure 1. There was a nonsignificant negative correlation between the maternal hair Pb level and gestational age (r=-0.01 95% confidence interval [CI] -0.259 to 0.218, p>0.05), see Table 3. We categorized the hair Pb level by a cutoff of 1.45 ppm. For the group with Pb≥1.45 ppm, the crude odds ratio (OR) of 1.46 (95% CI 0.56–3.79) for PTB, but the result was not significant, see Table 4. Multivariate analysis showed that Pb could be a risk factor for PTB, although the adjusted OR was not significant (24.69, 95% CI 0.93–653.82, p>0.05), see Table 5.

Table 2. Pb, TNF-α and IL-6 measurement in all subjects

Variables	Mean±standard deviation; median (min-max)	p-value[a]
Maternal serum IL-6 (pg/ml)	18.28±19.6; 10.77 (0.64–95.05)	0.00
Maternal serum TNF-α (pg/ml)	23.02±27.82; 10.02 (1.48–131.46)	0.00
Maternal hair Pb level (ppm)	1.49±0.21; 1.5 (1.1–2)	0.01

Note: [a]Kolmogorov–Smirnov test. IL-6 and TNF-α measured with ELISA; maternal hair Pb was measured with total reflection X-ray fluorescence

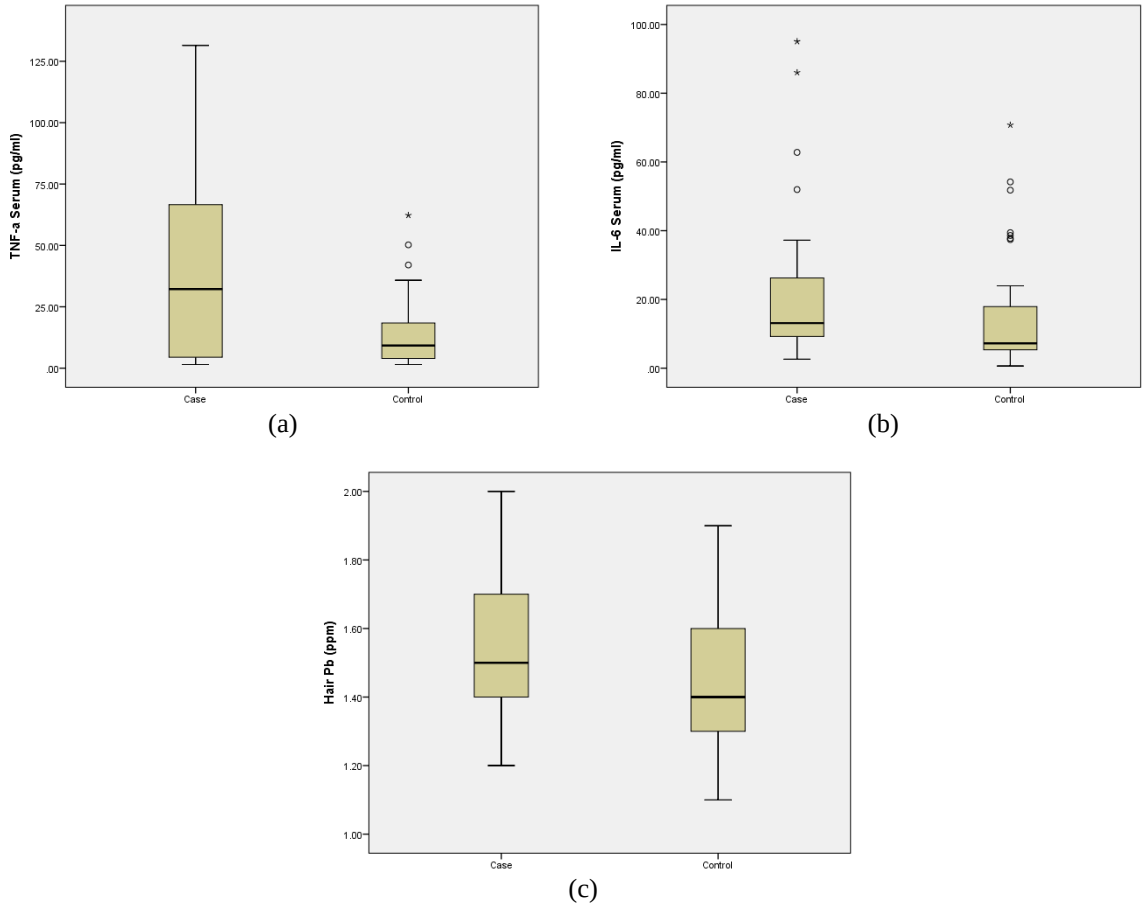

Figure 1. Comparison of (a) tumor necrosis factor-alpha (TNF-α), (b) interleukin 6 (IL-6), and (c) lead (Pb) levels between the case and control groups. The Mann–Whitney test revealed significantly higher IL-6 and TNF-α levels, and a slightly higher maternal hair Pb level in the case compared with the control group

Table 3. Correlation of Pb, TNF-α, IL-6, and gestational age

Variable	IL-6	TNF-α	Gestational age
Pb	r=-0.015[a]; p=0.897	r=-0.02[a]; p=0.893	r=-0.010[a]; p=0.936
IL-6		r=-0.031[a]; p=0.796	r=-0.157[a]; p=0.186
TNF-α			r=-0.233[a]; p=0.049*

Note: r=coefficient correlation; a=Spearman test; *p<0.05

Table 4. Assessing Pb, IL-6, and TNF-α levels as risk factors for PTB

Variables		N (%)		p-value	Crude odds ratio (95% confidence interval)
		Case (N=28)	Control (N=44)		
Maternal hair Pb	Pb<1.45 ppm	12 (42.9%)	23 (52.3%)	0.436[a]	1.46 (0.56–3.79)
	Pb≥1.45 ppm	16 (57.1%)	21 (47.7%)		
Maternal serum IL-6	IL-6<9 pg/ml	6 (21.4%)	24 (54.5%)	0.005[a]	4.4 (1.49–12.96)*
	IL-6≥9 pg/ml	22 (78.6%)	20 (45.5%)		
Maternal serum TNF-α	TNF-α<27 pg/ml	12 (42.9%)	40 (90.9%)	0.00[b]	13.33 (3.74–47.55)*
	TNF-α≥27 pg/ml	16 (57.1%)	4 (9.1%)		

Note: [a]Chi-square test; [b]Fisher's exact test; *p<0.05; The cut-off of Pb, IL-6, and TNF-α were determined by ROC analysis

Table 5. PTB risk factor

Variables	Coefficient	Standard error	p-value	Odds ratio	95% confidence interval	
					Min	Max
Interleukin 6≥9 pg/ml	3.106	1.000	0.002	22.33*	3.144	158.534
Tumor necrosis factor-alpha ≥27 pg/ml	3.744	1.063	0.000	42.25*	5.256	339.607
Hemoglobin	-0.842	0.391	0.031	0.43*	0.200	0.927
Spouse's smoking frequency	0.243	0.082	0.003	1.28*	1.087	1.496
Maternal lead level	3.206	1.672	0.055	24.69	0.932	653.819

Note: *Variables had significant results based on multivariate logistic regression

Pb is a heavy metal that can trigger toxic effects in organisms and affect immunomodulators [19]. Pb exposure in humans increases proinflammatory cytokines, suggesting possible Pb interference in inflammation [20]. An *in vitro* study with mononuclear cells from human peripheral blood showed increased stimulation of TNF-α and IL-6 at a high Pb dose of 150 ppm [21]. Based on a study that added 4–40 ppm Pb ions to peritoneal adherent cells and lymph cells, Pb interferes with the production of proinflammatory cytokine regulation. In that study, IL-6 and TNF-α levels were significantly lower compared with untreated cultured cells [22]. Pb exposure in workers for 40 days significantly increases Pb blood levels with a positive correlation with IL-6 levels [23]. Those findings demonstrate how the exposure dose and duration impact the immunomodulating effect.

Data analysis of the association between Pb exposure and PTB revealed an increased risk from both bivariate and multivariate tests, although the findings were not significant. Similarly, there was not a significant relationship between the Pb level and the IL-6 or TNF-α level. The median hair Pb level in the present study was 1.5 ppm, substantially lower than the World Health Organization (WHO) threshold of <12 ppm. This level is lower than earlier studies in Indonesia, suggesting that the Pb level may need to be higher to demonstrate the harmful effects of Pb exposure [21], [24], [25]. Previous studies in the Chinese and Japanese populations similarly demonstrated a slightly higher trend regarding the Pb in women with PTB, although the low Pb levels did not show a toxic association with PTB [26], [27]. The trace elements and micronutrients that are known as protective factors in pregnancy could influence the heavy metals pathology in PTB [26], [28], [29].

3.3. Analysis of Serum IL-6 and TNF- α Levels in Mothers with PTB

The median IL-6 serum level in all subjects was 10.77 (0.64–95.05) pg/ml. There was a significant difference between the case and control groups, in which the case had a higher median, 13.11 (2.6–95.05) versus 7.23 (0.64–70.76) $p<0.05$, see Figure 1. There was a nonsignificant negative correlation between the serum IL-6 level and gestational age ($r=-0.157$; $p>0.05$). A serum IL-6 level ≥9 pg/ml could be a risk factor for PTB, with a crude OR of 4.4 (95% CI 1.49–12.96, $p<0.05$) and an adjusted OR of 22.33 (95% CI 3.14–158.53).

The median serum TNF-α level in all subjects was 10.02 (1.48–131.46) pg/ml. The TNF-α level was significantly higher in the case group, which had a median of 32.2 (1.48–131.46) versus 9.22 (1.48–62.29) $p<0.05$, see Figure 1. There was a significant negative correlation between TNF-α and gestational age ($r=-0.233$, $p<0.05$). A TNF-α level ≥27 pg/ml could be a risk factor for PTB, with a crude OR of 13.33 (95% CI 3.74–47.55, $p<0.05$) and an adjusted OR of 42.25 (95% CI 5.26–339.61).

TNF-α is part of cellular immunity and contributes to PTB [30]. IL-6 plays a role in humoral immunity and is involved in labor and preterm physiology [31], [32]. It is produced by fibroblasts, keratinocytes, monocytes, macrophages, T cells, endometrial stromal cells, decidual cells, the amnion, and the chorion, and one of its triggering factors is TNF-α [31], [32]. *In vivo* and *in vitro* studies suggest that IL-6 and TNF-α promote PTB and premature membrane rupture through the production of a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9) [33]. IL-6 and TNF-α also disrupt trophoblast invasion and alter placental functions which induce preeclampsia and relate to PTB [34].

Proinflammatory cytokines induce the labor process. Uterine activation occurs during labor, followed by myometrial contractions that ripen and soften the cervix until the membranes are torn [35]. The uterine activation protein (UAP), leukocyte invasion, and a sterile, inflammatory-like process that leads to parturition (including damage-associated molecular patterns) are all activated in response to proinflammatory signals [35]. The influx of leukocytes to the cervix releases proteolytic enzymes that aid in remodeling the cervix until dilatation occurs [36]. Proinflammatory cytokines stimulate the synthesis of prostaglandins and metalloproteinases that lead to early labor by inducing membrane rupture, cervix maturation, and uterine contractions [30].

3.4. Analysis of other variables that affect PTB

Multivariate logistic regression showed that the spouse's smoking frequency and maternal hemoglobin may influence PTB. The spouse's smoking frequency could increase the risk of PTB (OR 1.28, 95% CI 1.1–1.5), while maternal hemoglobin could be a protective factor against PTB (OR 0.43, 95% CI 0.2–0.93). Smoking is known to have negative health impacts, particularly during pregnancy [37]. Passive smoking—breathing in cigarette smoke—may lead to severe adverse effects [38]. A low hemoglobin level, a condition known as anemia, is associated with an increased risk of stillbirth, PTB, and low birth weight [39], [40]. The frequency of smoking significantly increased the risk of PTB in the final multivariate analytic model. The hemoglobin level has the potential to be a substantial protective factor.

4. CONCLUSION

Maternal hair Pb levels, maternal serum IL-6 and TNF-α levels, and the spouse's smoking frequency increase the risk of PTB. However, maternal hemoglobin serves as a protective factor. The low maternal hair Pb level in the study population might have prevented the ability to observe the toxic effect of Pb. Future investigations should focus on other heavy metals and trace elements that could act as confounding factors in at-risk subjects to determine the toxic effects of Pb on fetal and maternal health.

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