RESEARCH ARTICLE

Urinary PYD/Creatinine Ratio Has Negative Correlation to Serum 25(OH)D and Positive Correlation to Chronic Lead Exposure Index

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Abstract

BACKGROUND: The burden of disease due to lead exposure continues to increase. Lead interferes with 25(OH)D hydroxylation and calcium transport, increasing osteoclastic activity and bone resorption. Pyridinoline crosslinks (PYD), as an indicator of bone damage, can be seen earlier compared to imaging changes. Therefore, it is necessary to determine the correlation between serum 25(OH)D levels and the urinary PYD/creatinine ratio in workers exposed to lead, since up to now, there are only limited studies related to it.

METHODS: This cross-sectional study involved 104 workers exposed to lead, selected from parents whose children had blood lead levels above 10 µg/dL. Questionnaires and physical examination were performed to obtain characteristic data from subjects. Data regarding blood lead levels, serum 25(OH)D levels, urinary PYD levels, and urinary creatinine levels were also obtained from various laboratory methods.

RESULTS: Most subjects (86.5%) had inadequate serum 25(OH)D. Median blood lead levels was 6.3 (1.2-35.5) µg/dL, chronic lead exposure index was 35.3 (1.2-535.8) years µg/dL, serum 25(OH)D levels was 22 (8-52) ng/mL, and urinary PYD/creatinine ratio was 5.3 (3.6-28.1)×10-6. There was a significant negative correlation between serum 25(OH)D levels and urinary PYD/creatinine ratio in workers exposed to lead. There was also a significant positive correlation between chronic lead exposure index and the urinary PYD/creatinine ratio.

CONCLUSION: Since urinary PYD/creatinine ratio is correlated with serum chronic lead exposure index and serum 25(OH)D levels, it suggests that pyridinoline might be a potential biomarker to detect bone metabolism disorder due to the chronic lead exposure. Vitamin D adequacy is also an important factor in preventing bone metabolism disorder amidst chronic lead exposure.

KEYWORDS: 25(OH)D, bone resorption, pyridinoline, lead, worker

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Introduction

The Institute for Health Metrics and Evaluation estimatFes that long-term health impacts from lead exposure contributed to the global burden of disease with disability-adjusted life years (DALYs) of 21.7 million in 2019.(1) The national average blood lead levels (BLL) was 3.2 µg/dL and DALYs related to lead exposure in Indonesia in 2019 were 711,530 years.(2,3) National Institute for Occupational Safety

and Health found that approximately 94% of adults with elevated BLLs were exposed to lead from the workplace, while data related to workers exposed to lead in Indonesia is still unclear.(4) There is no acceptable level of lead exposure for the body, according to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC).(5,6) The determination of BLL ≥5 µg/dL by WHO is a reference for initiating clinical intervention, and the determination of BLL ≥3.5 µg/dL by CDC is a definition for being considered a case in a surveillance program.(6)

Studies in mice showed that lead exposure disrupts 25(OH)D hydroxylation in the kidneys, decreasing 1,25(OH)2D synthesis.(7) Parathyroid hormone (PTH) is elevated when there is insufficient vitamin D, which reduces the body's ability to absorb calcium.(7,8) Increased osteoclastic activity by PTH will increase bone resorption, create local foci of bone weakness, and decrease bone mineral density thereby increasing the risk of osteopenia and osteoporosis.(7) Low serum 25(OH)D levels have an increased risk of hip fracture, and this effect was clearly seen when serum 25(OH)D levels were <60 nmol/L.(9) The luminal calcium transporter 1 (CaT1), which plays a role in the calcium absorption process through active transport in the intestine, has the same high affinity for both calcium and lead so that ingested lead exposure can cause impaired calcium absorption due to the competition that occurs.(10)

Pyridinoline crosslinks (PYD) is a pyridinium covalent crosslink that bridges collagen peptides and mechanically stabilizes collagen molecules. PYD is produced from the breakdown of collagen during bone resorption and is excreted in the urine. Its levels reflect the degradation of mature, cross-linked collagen. PYD is stable in urine samples stored at room temperature for several weeks and at -20°C for years. The amount of PYD in the urine can change based on its concentration. One waste product that is expelled from urine at a steady pace is creatinine. A more accurate evaluation of PYD excretion is obtained by accounting for urine concentration variations by measuring PYD relative to creatinine.(11,12) It is currently known that the gold standard for assessing bone mass density (BMD) is dual-energy X-ray absorptiometry (DXA). However, repeated DXA testing in many asymptomatic patients would be expensive. Bone biochemical markers are more economical and convenient than BMD testing, and changes in bone biochemical markers occur earlier than imaging changes.(13) A study conducted in patients with small bowel resection found a negative and significant correlation between serum 25(OH)D levels and PYD.(14) Other studies on serum 25(OH)D levels and urinary PYD/creatinine ratio in workers exposed to lead are still very limited, so this study was conducted to see the correlation between serum 25(OH)D levels and urinary PYD/creatinine ratio in workers exposed to lead in Indonesia.

Methods

Study Design and Subjects Recruitment

This cross-sectional study was a part of the study entitled Determinant Factors of Children's Blood Lead Levels in Java, Indonesia (further referred as the Primary Study) (15), which used high lead exposure study area from the with the technical assistance from Pure Earth (New York, NY, USA). Using the "Toxic Site Identification Program" (TSIP), 95 locations on the Java and Sumatera islands were evaluated for their potential to pollute the environment with lead. Of these, 89.6% are engaged in operations pertaining to the recycling of spent batteries, and 67 of them were situated on Java Island.(16)

The samples in the Primary Study were children (15), but the target population for this current study was all workers exposed to lead, selected from parents whose children had blood lead levels above 10 µg/dL in the Primary Study (17). The subjects were selected using a purposive method with the inclusion criteria were male workers aged 30 to 60 years, had a minimum of 3 months of length of service, and had normal liver and kidney function based on the results of blood tests. The exclusion criteria were workers with a history of food absorption disorders within 3 weeks prior to study, and workers who were undergoing radiotherapy.

The sample size was calculated using the correlation formula (r=0.34).(14) Based on this formula, the minimum number of samples required was 66 people. The results of the search conducted from the Primary Study showed that there were 104 people who met the inclusion and exclusion criteria, thus meeting the minimum number of samples required. The protocol of this study was approved by the Health Research Ethics Committee of Dr. Cipto Mangunkusumo General Hospital/Faculty of Medicine, Universitas Indonesia (No. 866/UN2.F1/ETIK/PPM.00.02/2023), and all subjects had signed informed consent prior to the study.

Questionnaires and Physical Examination

Global Physical Activity Questionnaire (GPAQ) and Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) were used to obtain information regarding the subject characteristics, including physical activity and nutrient

intake data. The GPAQ used in this study had been translated into Indonesian and had been tested the validity with a validity value >0.296 (R table) and reliable with a Cronbach's alpha value >0.88.(18) The SQ-FFQ used in this study had been used nationally and was recommended by the Ministry of Health Republic Indonesia in cohort studies of non-communicable disease risk factors.(19) The chronic lead exposure index was calculated based on the length of service (years) multiplied by blood lead level (mg/dL). Meanwhile, the physical examination was performed to obtain data regarding subjects' body mass index.

Samples Collection and Preparation

Blood samples were withdrawn from the middle cubital vein of subjects. A total of 2×3 mL was placed into an ethylenediaminetetraacetic acid (EDTA) vacutainer for examination of blood lead levels, liver function, and kidney function. A 5 mL blood sample was placed into a serum separator tube (SST) vacutainer for examination of 25(OH) D levels. A 60 mL morning urine sample was obtained for examination of pyridinoline crosslinks and creatinine. The samples were kept at 4°C, and transferred to a laboratory using freezers for the cool chain. Quality assurance and control procedures were used throughout the analysis to ensure the accuracy and precision of the results.

Blood Lead Levels Analysis

Blood lead levels were measured using the inductively coupled plasma-mass spectrometry (ICP-MS) method. ICP-MS method was the current gold standard with high sensitivity for determining blood lead levels.(18) The blood samples were prepared for analysis by dilution with 10% of nitric acid (Fisher Scientific®, Loughborough, UK) in deionized water.

Serum 25(OH)D Levels Analysis

Serum 25(OH)D levels were measured using the electrochemiluminescence assay (ECLIA) method with the Eclesys® Vitamin D total III Kit (Roche Diagnostic, Basel, Switzerland) according to the kit manual. ECLIA method was a widely available, rapid, and inexpensive assay for analyzing serum 25(OH)D levels.(20)

Urinary Pyridinoline Crosslinks Levels Analysis

Urinary pyridinoline crosslinks levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. ELISA method was a widely used and convenient method for analyzing urinary pyridinoline crosslinks.(21) The urine samples were prepared for analysis by collecting urine into aseptic tubes and collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm.

Urinary Creatinine Levels Analysis

Urinary creatinine levels were measured using the most frequently used rapid, and inexpensive method, colorimetric assay.(22) StressXpress® Urine Creatinine Detection Kit (Cat. No. SKT-200; StressMarq Biosciences, Victoria, BC, Canada) was used. The urine samples were prepared for analysis by diluting 1:20 with deionized or distilled water by taking one part of urine and adding to nineteen parts of water to obtain accurate results. Any urine samples with creatinine concentrations outside the standard curve range should be diluted further with water to obtain readings within the standard curve.

Statistical Analysis

Subject characteristic analysis was conducted to obtain the central tendency or frequency distribution of the variables of age, type of work, length of service, working duration, history of sun exposure sufficiency, physical activity, body mass index, nutritional status, vitamin D intake, calcium intake, protein intake, blood lead levels, chronic lead exposure index, serum 25(OH)D levels, and urinary PYD/creatinine ratio. The study used a Kolmogorov-Smirnov Test to analyze the variable data distribution. The Central tendency value of variables which were normally distributed are presented in mean±standard deviation (SD), otherwise presented in median (min-max). This study used a correlation analysis to obtain correlation coefficients of chronic lead exposure index, and serum 25(OH)D levels to the urinary PYD/creatinine ratio. The Pearson Correlation analysis was used to analyze the normally data distributed data variables, and Spearman-Rho Correlation test was used to analyze the non-normally distributed ones using IBM SPSS Statistics ver. 26 software (IBM Corporation, Armonk, NY, USA).

Results

Data from 141 subjects involved in the Primary Study was obtained for further analysis. A total of 27 subjects did not have stored morning urine samples. In addition, 10 subjects did not meet the study criteria, resulting in a total of 104 subjects (Figure 1). The distribution of each research area was 24 subjects from Tegal Regency, 19 subjects from Tangerang Regency, 16 subjects from Surabaya City, and 45 subjects from Bogor Regency.

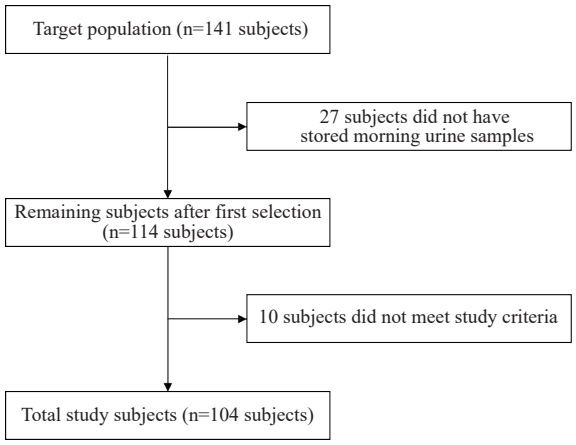

Figure 1. Chart of the study subjects selection.

The median age of the subjects was 39.5 (30-58) years, the median length of service was 7 (1-43) years, the median working duration was 8 hours, the proportion of subjects had outdoor type of work (47.1%), more than half subjects did not have a history of sun exposure sufficiency (58.7%), subjects did the moderate physical activity (52.9%), the median body mass index was 21.5 kg/m^2, the proportion of subjects had normal nutritional status (46.2%). The median vitamin D intake was 1.3 (0.1-24.7) µg, calcium intake was 360.6 (50.5-1425.6) mg, protein intake was 0.9 (0.3-2.9) g/kg BW, and two-third (66.3%) of subjects had sufficient protein intake status. The study also found that the median of blood lead levels was 6.3 (1.2-35.5) µg/dL, the chronic lead exposure index was 35.3 (1.2-535.8) years µg/dL, serum 25(OH)D levels was 22 (8-52) ng/mL, and median urinary PYD/creatinine ratio was 5.3 (3.6-28.1)×10^{-6} (Table 1). Interestingly, almost all of study participants (86.5% of subjects) had inadequate serum 25(OH)D levels, either insufficiency or deficiency.

Correlation of Urinary PYD/creatinine with Other Parameters

The correlations between the urinary PYD/creatinine ratio with other parameters including serum 25(OH)D levels, chronic lead exposure index, and subjects' characteristics were analyzed (Table 2). Other than with serum 25(OH) D levels and chronic lead exposure index, there was no significant correlation between urinary PYD/creatinine and the variables of age, working duration, body mass index, vitamin D intake, calcium intake, as well as protein intake (p>0.05).

The results of correlation analysis showed that there was a significant positive correlation between chronic lead exposure index and the urinary PYD/creatinine ratio (r=0.21, p=0.036) (Figure 2). It was also found that there was a significant negative correlation between serum 25(OH)D levels and the urinary PYD/creatinine ratio in workers exposed to lead (r=-0.39, p<0.001) (Figure 3).

Table 3 showed further analysis by including independent variables that had p≤0.200 in the previous bivariate analysis shown in Table 2. The independent variables included in this further analysis were working duration, body mass index, protein intake, chronic lead exposure index, and serum 25 (OH)D levels. The results of the further analysis showed that the adjusted determination coefficient value (adjusted R^2) was 0.110.

Discussion

The urinary PYD/creatinine ratio of the subjects in this study ranged from 3.6×10^{-6} to 28.1×10^{-6} and the median was 5.3×10^{-6}. Subjects with high urinary PYD/creatinine ratio values (>21.8×10^{-6}) indicated that there was an increase in bone resorption activity beyond the average of normal people.(23) The results of this study showed that urinary

Table 1. Characteristics of subjects (n=104).

Characteristic	n (%)	Median (Min-Max)
Age (years)		39.5 (30-58)
Length of service (years)		7 (1-43)
Working duration (hours)		8 (5-12)
Type of work		
Outdoor	49 (47.1)	
Indoor	47 (45.2)	
Combination	8 (7.7)	
History of sun exposure sufficiency		
Yes	43 (41.3)	
No	61 (58.7)	
Physical activity		
Light	5 (4.8)	
Moderate	55 (52.9)	
Vigorous	44 (42.3)	
Body mass index		21.5 (16.1-34.2)
Nutritional status		
Underweight	13 (12.5)	
Normal	48 (46.1)	
Overweight	19 (18.3)	
Obesity	24 (23.1)	
Vitamin D intake (µg)		1.3 (0.1-24.7)
Calcium intake (mg)		360.6 (50.5-1,425.6)
Protein intake (g/kg BW)		0.9 (0.3-2.9)
Protein intake status		
Sufficient	69 (66.3)	
Insufficient	35 (33.7)	
Blood lead levels (µg/dL)		6.3 (1.2-35.5)
Chronic lead exposure index (years µg/dL)		35.3 (1.2-535.8)
Serum 25(OH)D levels (ng/mL)		22 (8-52)
Urinary PYD/creatinine ratio		5.3 (3.6-28.1)×10^{-6}

Table 2. Correlation urinary PYD/creatinine ratio and other parameters (n=104).

Variable	Urinary PYD/Creatinine Ratio (r value)	*p*-value
Age (years)	-0.03	0.768
Working duration (hours)	0.16	0.111
Body mass index (kg/m^2)	-0.15	0.140
Vitamin D intake (µg)	0.02	0.827
Calcium intake (mg)	0.10	0.296
Protein intake (g/kgBW)	0.15	0.139
Chronic lead exposure index (years µg/dL)	0.21	0.036*
Serum 25(OH)D levels (ng/mL)	-0.39	<0.001*

*significant if $p<0.05$.

PYD/creatinine ratio is correlated with both chronic lead exposure index and serum 25(OH)D level, even though in opposite pattern.

The median of blood lead levels in this study was higher than the national average report of blood lead levels in Indonesia in 2019, which was 3.2 µg/dL.(2) This certainly needs special attention considering the high lead exposure in the subjects of this study, while World Health Organization (WHO) has decided that clinical intervention is needed in findings of blood lead levels ≥5 µg/dL. In addition, blood lead levels ≥10 µg/dL were associated with increased metabolism and bone loss, caries, tooth loss.(5,24) Inhalation and ingestion of lead can both result in human exposure. In the community at large, inhalation routes are less common than in occupationally exposed groups.(25) The length of service as the proxy of length exposure was one of the things that needs to be considered to explain chronic lead exposure in subjects. Approximately 95% of lead in circulation is bound by red blood cells and has a half-life of approximately 25 to 30 days.(26) Exposure period was significantly associated with bone turnover biological markers. Workers with longer service time will be at greater risk of lead exposure.(27) The chronic lead exposure index used in this study could explain how chronic lead exposure was related to urinary PYD/creatinine ratio.

Calcidiol or 25(OH)D was used in this study because it is the form of vitamin D that circulates most in the blood and has a long half-life, which is around 10 days to 3 weeks.(28) Referring to the classification of adequate serum 25(OH)D levels, out of 104 subjects in this study, only 14 subjects was adequate (serum 25(OH)D levels >29 µg/dL). The remaining 90 subjects were classified as insufficiency and deficiency. The median of 22 ng/mL was classified as insuficiency.(29) A study conducted on a group of workers exposed to lead also showed low mean serum 25(OH)D levels (28.2±10.8 ng/mL) and mean blood lead levels was

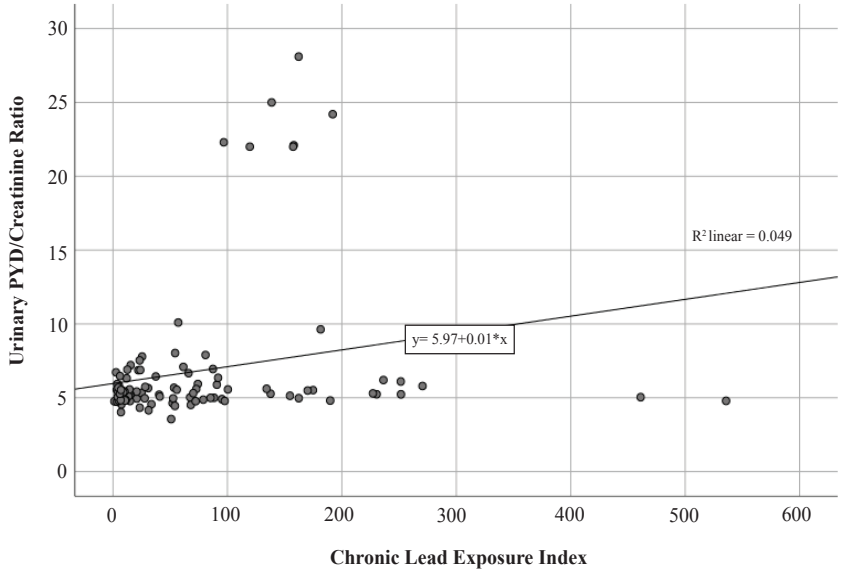

Figure 2. Correlation of chronic lead exposure index with urinary PYD/ creatinine ratio.

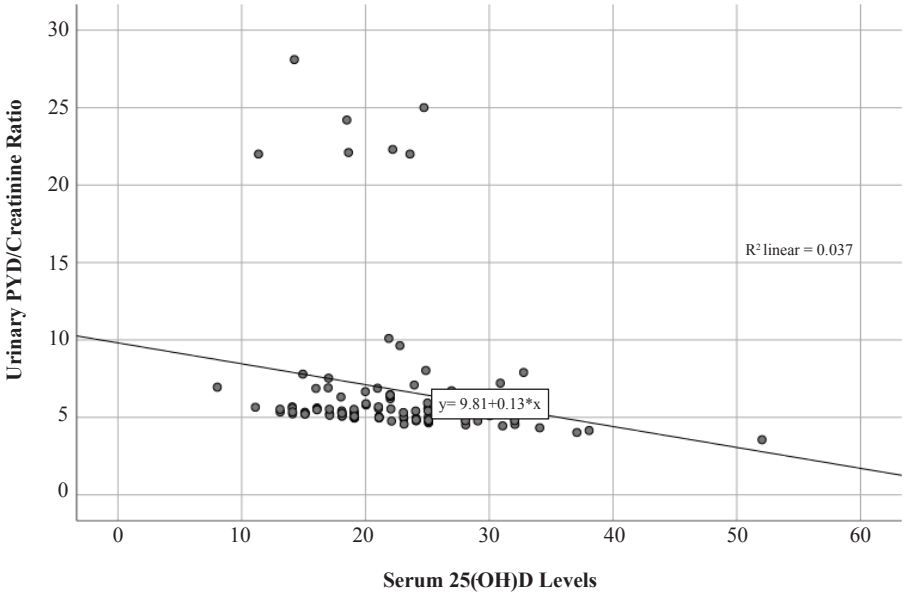

Figure 3. Correlation of serum 25(OH)D levels with urinary PYD/creatinine ratio.

38.0±19.9 µg/dL. Likewise, the control group with mean blood lead levels was 2.3±1.2 µg/dL had mean serum 25(OH)D levels that were also low (37.0±7.8 ng/mL). (30) This study found that all subjects had serum 25(OH) D levels <60 ng/mL. Low serum 25(OH)D levels have an increased risk of hip fracture, and this effect is clearly seen when serum 25(OH)D levels are <60 ng/L.(9) The low median serum 25(OH)D levels in subjects in this study could be caused by several things, such as low exposure to sunlight, low vitamin D intake, and lead exposure. This is supported by the results of this study which show that more than half subjects did not have a history of sun exposure sufficiency, have low median intake of vitamin D, and have long working duration or service period which increase their exposure to lead.

Although the results of this study do not show significant results, but the correlations between low bone mass density and other factors might also be considered. As many as 13% of the subjects in this study had underweight nutritional status. Low body mass index was associated with low bone mass density, reduced soft tissue, and muscle weakness.(31) The median intake of vitamin D of subjects in this study was still far below the nutritional adequacy rate based on Indonesian Recommended Dietary Allowances (*Angka Kecukupan Gizi* = AKG) for vitamin D in the 10-64 years age group, which is 15 µg/day.(32) It was found that the study subjects rarely consumed food sources of vitamin D in the past month. Subjects tended to consume food with low vitamin D content, such as mackerel, tuna, and chicken egg yolks. Medium intake of calcium of subjects in this study was also still below the adequate calcium intake according to the AKG, which is 1,000-1,200 mg per day.(32) However, refer to the daily protein requirement of each subject, which was 0.8 g/kgBW, among 66.3% of the study subjects had sufficient protein intake status.

Other study of workers exposed to lead found that PYD and other bone resorption indicators were higher. Additionally, the study discovered that workers with higher BLL values also had higher PYD levels.(27) Another study

Table 3. Factors associated with urinary PYD/creatinine ratio (n=104).

Variable	Parameter Estimate	Standard Error	95% of CI for Parameter	*p* -value
Intercept	12.80	4.12	4.61–21.00	0.003*
Working duration	-0.17	0.25	-0.67–0.34	0.515
Body mass index	-0.17	0.11	-0.40–0.05	0.134
Protein intake	1.53	0.82	-0.10–3.17	0.065
Chronic lead exposure index	0.01	0.01	0.00– 0.02	0.024*
Serum 25(OH)D levels	-0.14	0.07	-0.27–(-0.01)	0.033*

*significant if $p<0.05$.

in patients with small bowel resection conducted also found that there was a negative and significant correlation between serum 25(OH)D levels and urinary PYD (r=-0.350, *p*=0.030).(14) In addition, low 25(OH)D levels were also significantly associated with low spine and hip BMD z-scores. Vitamin D has many functions, including its role in calcium metabolism and bone health. Lead exposure disrupts 25(OH)D hydroxylation in the kidneys.(8,27) PTH is elevated when there is insufficient vitamin D, which reduces the body's ability to absorb calcium.(7,8) Increased osteoclastic activity by PTH will increase bone resorption, create local foci of bone weakness, and decrease bone mineral density thereby increasing the risk of osteopenia and osteoporosis.(7) This study showed the importance of education on prevention and management of lead exposure, as well as maintaining serum 25(OH) D levels in workers to be sufficient through vitamin D intake, physical activity, adequate sun exposure, vitamin D supplementation if necessary, and examination for serum 25(OH)D levels regularly. Studies on other natural sources as a way to overcome the effects of lead exposure are also needed, for example a study examining the benefits of using paddy leaves extract as a hepatoprotective agent against liver damage due to lead exposure.(33) Based on the results of this study, PYD is a potential biomarker for early detection of bone resorption disorder in occupational medicine practice. In addition, the use of biomarkers is more economical and convenient than BMD testing, and changes in biomarkers occur earlier than imaging. Further studies are needed to elucidate the mechanisms of urinary PYD/creatinine ratio as a link with serum 25(OH)D levels in lead exposed worker.

Conclusion

Since urinary PYD/creatinine ratio is correlated with serum chronic lead exposure index and serum 25(OH)D levels, it suggests that pyridinoline might be identified as a possible biomarker for detecting bone metabolism disorders caused by chronic lead exposure. The use of pyridinoline as a biomarker in this study showed that there was a mechanism that explained the relationship between lead exposure and serum 25(OH)D levels on bone metabolism. Adequate serum 25(OH)D levels play a crucial role in avoiding bone metabolism disorders caused by prolonged exposure to lead. This implied that the need to maintain adequate serum 25(OH)D levels in workers exposed to lead was as important as monitoring blood lead levels.

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Authors Contribution

All authors participated in developing the research idea, interpreting the results, and preparing the manuscript. SSH and MM conducted data acquisition and analysis. NM, NRM, and M contributed to critically revising the manuscript.

References

1. Zhou N, Huang Y, Li M, Zhou L, Jin H. Trends in global burden of diseases attributable to lead exposure in 204 countries and territories from 1990 to 2019. Front Public Health. 2022; 10: 1036398. doi: 10.3389/fpubh.2022.1036398.
2. Leadpollution.org [Internet]. Lead Country Statistics; ©2019 [cited 2023 Nov 18]. Available from: https://leadpollution.org/?indicator=DALYs_IHME_2019.
3. Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH, *et al.* Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018; 392(10159): 1923-94.
4. Shaffer RM, Gilbert SG. Reducing occupational lead exposures: strengthened standards for A healthy workforce. Neurotoxicology. 2018; 69: 181-6.
5. World Health Organization. WHO Guideline for Clinical Management of Exposure to Lead. Geneva: World Health Organization; 2021.
6. Ruckart PZ, Jones RL, Courtney JG, LeBlanc TT, Jackson W, Karwowski MP, *et al.* Update of the blood lead eeference value – United States, 2021. Morb Mortal Wkly Rep. 2021; 70(43): 1509-12.
7. Nair R, Maseeh A. Vitamin D: The sunshine vitamin. J Pharmacol Pharmacother. 2012; 3(2): 118-26.
8. Rahman A, Al-Awadi AA, Khan KM. Lead affects vitamin D metabolism in rats. Nutrients. 2018; 10(3): 264. doi: 10.3390/nu10030264.
9. Lv QB, Gao X, Liu X, Shao ZX, Xu QH, Tang L, *et al.* The serum 25-hydroxyvitamin D levels and hip fracture risk: A meta-analysis of prospective cohort studies. Oncotarget. 2017; 8(24): 39849-58.

10. Kordas K. The lead diet: Can dietary approaches prevent or treat lead exposure. J Pediatr. 2017; 185: 224-31.
11. Wahyudin F. Graf Tulang & Material Pengganti Tulang: Karakteristik dan Strategi Aplikasi Klinis. Surabaya: Airlangga University Press; 2018.
12. Macias I, Sevillano NA, Rodriguez CI, Infante A. Osteoporosis and the potential of cell-based therapeutic strategies. Int J Mol Sci. 2020; 21(5): 1653. doi: 10.3390/ijms21051653.
13. Wang GX, Li JT, Cai F, Huang BL, Fang ZB, Chu SF, *et al.* The correlation of two bone turnover markers with bone mineral density: A population-based cross-sectional study. BMC Musculoskelet Disord. 2023; 24: 675. doi: 10.1186/s12891-023-06613-5.
14. Haderslev KV, Jeppesen PB, Sorensen HA, Mortensen PB, Staun M. Vitamin D status and measurements of markers of bone metabolism in patients with small intestinal resection. Gut. 2003; 52(5): 653-8.
15. Mansyur M, Fitriani DY, Prayogo A, Mutiara A, Asep, Fadhillah R, *et al.* Determinant factors of children's blood lead levels in Java, Indonesia. Int J Hyg Environ Health. 2024; 261: 114426. doi: 10.1016/j.ijheh.2024.114426.
16. Pure Earth [Internet]. Toxic Sites Identification Program; ©2023 [cited 2023 Aug 31]. Available from: https://www.contaminatedsites.org/.
17. Putri EA, Anisa R, Sulistyowati E. Differences in knowledge levels and behavioral patterns to prevent type 2 diabetes mellitus after physical activity pattern counseling to lecture and academic staff in Malang University. eJKI. 2019; 8(2): 56-65.
18. Saldana VR, Fobil J, Basu N. Lead (Pb) exposure assessment in dried blood spots using total reflection X-ray fluorescence (TXRF). Environ Res. 2021; 198: 110444. doi: 10.1016/j.envres.2020.110444.
19. Ministry of Health Republic Indonesia. Kuesioner Survei Data Dasar: studi kohor faktor risiko penyakit tidak menular. Jakarta: Ministry of Health Republic Indonesia; 2010.
20. Zajic P, Heschl S, Schorghuber M, Filzmaier PS, Stojakovic T, Weixler V, *et al.* Vitamin D assessment in perioperative medicine and critical care. Wien Klin Wochenschr. 2021; 133(3): 79-85.
21. Arbault P, Grimaux M, Pradet V, Predaut C, Seguin P, Delmas PD. Assessment of urinary pyridinoline excretion with a specific enzyme-linked immunosorbent assay in normal adults and in metabolic bone diseases. Bone. 1995; 16(4): 461-7.
22. Bezinge L, Tappauf N, Richards DA, Shih CJ, deMello AJ. Rapid electrochemical flow analysis of urinary creatinine on paper: Unleashing the potential of two-electrode detection. ACS Sens. 2023; 8(10): 3964-72.
23. Fraser WD [Internet]. Pyridinoline/Creatinine Ratio; ©2011 [cited 2023 Dec 12]. Available from: https://pathlabs.rlbuht.nhs.uk/pyridinolinecreatinineratio_urine_c.htm.
24. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead. Atlanta: The Department; 2020.
25. Mulyana, Arif M, Muliaty D. An antioxidant marker, aminolevulinic acid dehydratase, was correlated with bone turnover activity. Indones Biomed J. 2012; 4(3): 180-5.
26. Ahmad SA, Khan MH, Khandker S, Sarwar AFM, Yasmin N, Faruquee MH, *et al.* Blood lead levels and health problems of lead acid battery workers in Bangladesh. Scientific World J. 2014: 2014: 974104. doi: 10.1155/2014/974104.
27. Ravibabu K, Barman T, Bagepally BS. Assessment of bone turnover biomarkers in lead-battery workers with long-term exposure to lead. Int J Occup Environ Med. 2020; 11(3): 140-7.
28. Sari DK, Sari LM, Laksmi LI, Farhat F, Daulay ER, Sandra F, *et al.* Serumal and salivary 25(OH)D and 1,25(OH)D levels of head and neck cancer patients. Indones Biomed J. 2021; 13(4): 337-443.
29. The Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. Chevy Chase: The Endocrine Society; 2011.
30. Batra J, Thakur A, Meena SK, Singh L, Kumar J, Juyal D. Blood lead levels among the occupationally exposed workers and its effect on calcium and vitamin D metabolism: A case-control study. J Family Med Prim Care. 2020; 9(5): 2388-93.
31. Park SY. Association of body mass index and fracture risk varied by affected bones in patients with diabetes: A nationwide cohort study. Diabetes Metab J. 2023; 47(3): 437-8.
32. Ministry of Health Republic Indonesia. Angka Kecukupan Gizi yang Dianjurkan untuk Masyarakat Indonesia. Jakarta: Ministry of Health Republic Indonesia; 2019.
33. Santosa B, Sunoko HR, Sukeksi A, Zulaikhah ST. IR begendit paddy leaves extract improves liver cell morphology and reduces the activity of transaminase enzymes after lead exposure in rat. Indones Biomed J. 2020; 12(3): 227-32.