Original Article

Relationship between Cadmium, Chromium, and Plumbum Levels with Hydrogen Peroxide: Detection of Vascular Oxidative Stress Due to Metal Exposure as a Risk Factor for Cardiovascular Disease

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Abstract

Introduction: An environment exposed to heavy metals has the potential to cause cardiovascular disease. This condition increases free radical formation, namely hydrogen peroxide (H2O2), contributing to vascular damage. This research aims to analyze the relationship between Cadmium, Chromium, and Plumbum levels with H2O2, as risk factors for cardiovascular disease (CVD).

Methods: The study design was a cross-sectional of 120 respondents living in an industrial area. The metal levels in the blood were measured using atomic absorption spectrophotometry and peroxide number analysis.

Result: The results of the chi-square test at a 5% significance level showed a meaningful relationship between Cr and H2O2 ($p<0,001$), Pb, and H2O2 ($p=0.019$). However, there was no significant relationship between Cd and H2O2 levels ($p=0.07$).

Conclusion: In conclusion, exposure to heavy metals (Cr and Pb) is a potential risk factor for cardiovascular disease through oxidative stress mechanisms.

Keywords: cardiovascular disease, environmental hazard, heavy metals pollution, hydrogen peroxide, the risk factor.

INTRODUCTION

Cardiovascular Disease (CVD) is the world-leading cause of death in developed and developing countries. CVD causes 17.9 million deaths per year, this is 31% higher than all causes of death in the world.[1] This disease has classic risk factors such as dyslipidemia, hypertension, obesity, smoking habits, and inactivity.[2] Gutiérrez-Bedmar et al. revealed that environmental factors are essential in CVD pathogenesis.[3]

Research has not explored much of the effects of metal exposure on CVD incidence through oxidative stress mechanisms. On the contrary, many experts have determined that oxidative stress in blood vessels causes cell damage and involves several signalling pathways.

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Oxidative stress is a condition where there is a disturbance in the Reactive Oxygen Species (ROS) balance, either temporary or permanent. ROS increases inflammation, DNA, protein damage, and fat peroxidation. Therefore, elevated ROS concentrations are involved in CVD pathogenesis.[4,3,5]

METHODS

This research used a cross-sectional study to measure Cd, Cr, Pb, and H2O2. The study used a purposive sampling method to select respondents living around the industrial area. The sample inclusion criteria were: (a) an individual was staying at least one year in an industrial area, (b) the maximum residence distance is within a radius of 5 km from the location, (c) have normal blood pressure (120/80 mmHg), (d) total serum cholesterol <200 mg/dL, (e) typical of body mass index (18.5–24.9 kg/m2), and have not a smoking habit. Researchers socialized the study, and the inclusion criteria of the sample used posters and social media to recruit participants. A total sample of 120 participants living in industrial areas met the study inclusion criteria.

Participants that met the inclusion criteria were required to have 5-6 mL of blood drawn to measure their Cd, Cr, Pb, and H2O2 levels. Blood collection was carried out in the Central Laboratory of Biomedical, Faculty of Medicine, Brawijaya University. A total of 5 ml of peripheral blood has been taken using the venous punction procedure, accommodated in an Ethylene Diamine Tetra Acetic (EDTA) tube. Blood was diluted with Axisxhield's Lymphoperm in the same volume ratio in a 15 ml tube, then centrifuged for 30 minutes at 6000 rpm. Blood samples were stored in a -20o C refrigerator for further testing.

The materials needed to measure those variables include Buck Scientific Model 210 VGP Flame Atomic Absorption Spectrometer instrument, equipped with Plumbum (Pb), Cadmium (Cd), and Chromium (Cr) cathode lamps, Atomic Absorption Spectrophotometry (AAS)-a flame in the range of Cr/Cd/Pb levels at 0.2 mg/L to 5.0 mg/L, and a wavelength of 357.9 nm, nitric acid (HNO3), a standard solution of Cr/Pb/Cd metal and ethylene gas (C2H2), chloroform, glacial acetic acid, potassium iodide (KI), 1% amylum indicator, and Na2S2O3.

Preparation of Metal Raw Solutions

The primary solution (Cr/Cd/Pb metal by 1000 mg/L) was pipetted into a 100 mL volumetric flask and adjusted with a diluent solution until the term marks.

Measurement of Metal Content by Atomic Absorption Spectrophotometry (AAS)

This test aimed to determine the metal levels in blood plasma following the SNI 06-6989.17-2004 standards using Atomic Absorption Spectrophotometry (AAS). The addition of nitric acid sought to dissolve metal analytes and remove the disturbing substances in the sample by electric heating, then marked by AAS using acetylene gas (C2H2). The preparation was to optimize the SSA tool according to the instructions for using the device. The researcher measured each work solution at a wavelength of 357.9 nm. A calibration curve was created to obtain a regression line equation, followed by measuring the prepared blood sample. The author calculated metal content using the following equation:

Total metal concentration, Cr/Cd/Pb (mg/L) = C x fp

with description:

C is the concentration obtained by the measurement results (mg/L);

fp is the dilution factor.

Percent of retrieval:

$$\% R = ((A - B)/C) \times 100 \%$$

With description:

A is the level of the spiked sample

B is the level of the unspiked sample

C is the standard level obtained (target level).

H2O2 Level Measurement

Making amylum 1%

The making of amylum 1% was carried out by weighing 1 gram of amylum, adding 100 ml of distilled water, and stirring and heating until the KI dissolved completely.

Making saturated potassium iodide (KI)

A KI was added into distilled water until it was insoluble, then filtered.

Measurement of H2O2 Levels using Peroxide Number Measurement (O_2^-/100gr).

This analysis aimed to determine the H2O2 levels in blood plasma. The blood plasma was 1 ml, then added with 12 ml chloroform and 18 ml of glacial acetic acid solution. Shook until the ingredients were dissolved, added 0.5 mL of saturated KI, and nodded for 1 min. Furthermore, add 30 mL of distilled water, then wait for up to 2 mins. Subsequently, 0.5 mL of 1% amylum indicator was added immediately and titrated with 0.1 N Na2S2O3. After these processes, the researcher measured with peroxide number the following equation:

Peroxide Number = (Volume Na2S2O3 x N Na2S2O3 X 1000)/sample weight (1)

Data Analysis

The calibration curve equation of the heavy metal and H2O2 indicated their concentration in blood plasma. The researchers calculated content from the regression equation obtained from the calibration curve and next classified it according to the normal and abnormal categories. Cd standard level in the blood was 0.03−0.12 μg/dl, Cr was ≤0.14 µg/dL, and Pb was ≤10 µg/dL.7,8 When the level of each heavy metal exceeded this limit, their blood levels were abnormal. Next, the researchers converted the amount

of H2O2 from numerical data into nominal, namely positive and negative H2O2. The H2O2 levels were positive when a peroxide number was detected (> 0 〖 O〗 _2^-/100gr), while it was negative when no peroxide number was seen (≤ 0 O_2^-/100gr).

Furthermore, the significance of the relationship between heavy metal levels (Cr/Cd/Pb) and oxidative stress events (H2O2) was determined through the Chi-square test on alpha 5%.

RESULT

Relationship between Heavy Metal (Cr/Cd/Pb) level with H2O2

Table 1 presented heavy metal levels (Cd/Cr/Pb) and H2O2 from respondent blood samples living in industrial areas. Based on Table 1, there was no relationship between Cd exposure and the incidence of oxidative stress. The significant association identified was the exposure to Cr and Pb with the incidence of oxidative stress.

Table 1: Cadmium, Chromium, and Plumbum Levels on the Incidence of Oxidative Stress and Non-Oxidative Stress

Heavy Metal Content	Incidence of oxidative stress n(f%)		p-value*
	Positive (> 0 O_2^-/100gr)	Negative (≤ 0 O_2^-/100gr)	
Cadmium			
Normal (0.03–0.12 µg/dl)	0 (0%)	16 (16.7%)	0.070
Abnormal (>0.12 µg/dl)	24(100%)	80 (83.3%)	
Chromium			
Normal (≤0.14 µg/dL)	2 (8.3%)	50 (52.1%)	<0.001
Abnormal (>0.14 µg/dL)	22 (91.7%)	46 (47.9%)	
Plumbum			
Normal (≤10 µg/dL.)	2 (8.3%)	34 (35.4%)	0.019
Abnormal (>10 µg/dL)	22 (91.7%)	62 (64.6%)	

* Calculated using the Chi-square Test at the significance level $\alpha \leq 5\%$

DISCUSSION

The reactive oxidant species (ROS) in the body were toxic because they caused molecularly (DNA, proteins, and lipids) and tissue damage and endothelial cell dysfunction in the body.[9,10] The reactive oxidant species (ROS) in the body were toxic because they caused molecularly (DNA, proteins, and lipids) and tissue damage and endothelial cell dysfunction.[9,10] The ROS also induced molecular damage because it triggered dyslipidemia and hyperglycemia.[10] H2O2 is a type of ROS produced by human cells through a one-electron reaction (free radicals) initially made by superoxide anion radicals.[11] The high H2O2 levels were indicators of vascular oxidative stress and played an essential role in CVD pathomechanism. The H2O2 levels were also elevated in the insulin resistance condition in diabetes mellitus mice.[12]

The high H2O2 levels in this research were found in 20% of the population with abnormal cadmium levels (> 0.12 µg/dL), 18.3% with abnormal chromium levels (> 0.14 µg/dL), and 18.3% with abnormal plumbum levels (> 0.14 µg/dL).

Relationship between Cadmium Levels with H2O2

Cadmium (Cd) is a metal that causes toxicity, and hypertension reduces kidney function and other organ problems.[13] In contrast to Tremellen's (2012) study, there is a relationship between oxidative stress and Cd toxicity.[14] In this study, 80 people (83.3%) had high Cd concentrations but had normal H2O2 levels. It was also contrary to other studies, which said that the higher the metal content, the higher the H2O2 level.[10] Based on the Chi-square test in this research, the Cd levels were not significantly related to H2O2 contents (p=0.07; p>0.05). This condition occurred because the Cd was not sufficient to stimulate the H2O2 formation in the blood.

Many researchers report that Cd is a heavy metal with a high level of exposure due to its distribution through water, soil, and even food.[15] Likewise, some researchers reported high Cd levels in industrial wastewater and nutrition, such as rice.[16] Cd affected the metabolism performance, especially in DNA damage and endothelial dysfunction, when the exposure was within an abnormal threshold.[17] Although there was no significant relationship between Cd and H2O2 levels, caution about Cd dangers to health still needs attention. This metal toxicity caused a loss of endothelial cell structure, leading to death due to thrombogenic events.[18] Cd also inhibited endothelial nitric oxide synthase and suppressed acetylcholine-induced vascular relaxation, which resulted in hypertension.[19] The continuous exposure stimulated excess cytokines production and induced CVD. The high Cd levels in the environment were caused by fossil fuels, burning metal ores, and waste. They were absorbed into agricultural soil, transferred into plants and food, and accumulated in various human organs.[20]

Relationship between Chromium Levels with H2O2

Gutiérrez-Bedmar et al. stated that blood samples, urine, hair, nails, and saliva could show chromium toxicity in the body.[3] In this research, the authors tested blood samples to calculate them. The standard Cr exposure limit in the body was <0.14 µg/dL in the blood.[8] The Chi-square analysis showed a significant relationship (p<0.001) between Cr and H2O2 levels. The association was in line with other studies on the relationship between Cr and CVD (5). Thus,

increased metal exposure in industrial areas causes an increase in Cr content. Therefore, those who live in these locations are very vulnerable to Cr pollution through the soil, water, and air.[21] This fact is in line with this study's results, which found that 91.7% of the population had abnormal levels of Cr (> 0.14 µg / dL) and H2O2.

In vitro and in vivo studies conducted by Stohs et al. showed that the cation of Cr metal-induced oxidative stress resulted in a macro-oxidative reduction of biological molecules. Cr underwent a redox cycle which increased ROS production, such as superoxide ions, hydroxyl radicals, and hydrogen peroxide.[16] These reactive oxygen species increased lipid peroxidation, excretion of urinary lipid metabolites, modulation of intracellular oxidation states, DNA damage, membrane damage, altered gene expression, and apoptosis. The condition indicated that Cr is a hazardous metal compound that generally affects the body's redox system and increases H2O2.

Relationship between Plumbum Levels with H2O2

Pb toxicity is often associated with heart and blood vessel damage with severe and deadly consequences, including hypertension and CVD.[22] The disruption of the balance between free radical production and the biological system's ability to detoxify reactive substances in the body triggered oxidative stress.[7, 23-25] Another research detected high Pb levels in factory workers in Korea caused by increased exposure to this metal in industrial areas.[25] The Chi-square analysis indicated a significant difference ($p<0.05$) between the Pb and H2O2 levels. This condition indicated that Pb contributed to CVD development as characterized by oxidative stress in blood vessels.

The Chi-square test showed a significant relationship between heavy metals (Cr, Pb) and biomarkers of oxidative stress (H2O2), with p-values of <0.001 and 0.019 for each metal ($p <0.05$). Therefore, Cadmium and Plumbum have a significant relationship in increasing oxidative stress biomarkers (H2O2) as a risk factor for cardiovascular disease. Furthermore, Pb or plumbum has neurotoxin and poisoning effects caused by the pollution from factory waste, leaded gasoline fuel, dust, old lead-based paint, cosmetics, and food consumed.[26,27] This condition puts the community at high risk of prolonged heavy metals poisoning.

CONCLUSION

There was a significant relationship between Cr and Pb with H2O2 levels in respondents living in industrial areas. However, there was no critical relationship between Cd and H2O2 levels. The exposure to heavy metals, especially Cr and Pb, triggered oxidative stress on the vascular system. These results reinforced that exposure to heavy metals, especially Cr and Pb, is the newest risk factor for cardiovascular disease. Therefore, the other researchers need to conduct a cohort study to determine these metals' exposure as an etiological factor for oxidative stress in cardiovascular disease. The study recommends developing efforts to prevent and control the negative impacts of industrial activities in residential areas.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest in this study

ETHICAL CLEARANCE

The Ethics Commission of Medicine Faculty, University of Brawijaya, approved this research with Number 014/EC/KEPK/2019.

AUTHOR CONTRIBUTION:

Conceptualization: KK; Validation: KK, TAW; Formal analysis and investigation: INC, SW, WO; Writing—original draft: INC, SW; Writing—review and editing; KK, TAW, INC; Supervision: KK, TAW. All authors read and agreed to the published version of the manuscript.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs). 2017. [Accessed on October 30, 2018]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Dahlöf B. Cardiovascular disease risk factors: epidemiology and risk assessment. Am J Cardiol. 2010; 105(1): 3A-9A. doi: 10.1016/j.amjcard.2009.10.007. PMID: 20102968.
3. Gutiérrez-Bedmar M, Martínez-González MÁ, Muñoz-Bravo C, Ruiz-Canela M, Mariscal A, Salas-Salvadó J, et al. Chromium Exposure and Risk of Cardiovascular Disease in High Cardiovascular Risk Subjects - Nested Case-Control Study in the Prevention with Mediterranean Diet (PREDIMED) Study. Circ J. 2017; 81(8): 1183-1190. doi: 10.1253/circj.CJ-17-0032. Epub 2017 Apr 8. PMID: 28392547.
4. Heriansyah T, Nurwidyaningtyas W, Sargowo D, Tjahjono CT, Wihastuti TA. Polysaccharide peptide (PsP) Ganoderma lucidum: a potential inducer for vascular repair in type 2 diabetes mellitus model. Vasc Health Risk Manag. 2019; 15: 419-427. doi: 10.2147/VHRM.S205996.
5. Bretón-Romero R, Lamas S. Hydrogen peroxide signaling in vascular endothelial cells. Redox Biol. 2014; 2: 529-34. doi: 10.1016/j.redox.2014.02.005.
6. Heriansyah T, Kumboyono K, Cesa FY, Wihastuti TA, Wisnasari S. Correlation Between Knowledge, Attitudes, and Behavior with Blood Cadmium Levels in the Population of Industrial Area. Indian Journal of Forensic Medicine & Toxicology 2021; January-March; 15 (1)
7. Alli LA. Blood level of Cadmium and lead in occupationally exposed persons in Gwagwalada, Abuja, Nigeria. Interdiscip Toxicol. 2015; 8(3): 146-50. doi: 10.1515/intox-2015-0022.
8. University of California San Francisco Health website. Chromium - blood test. 2019. [Accessed on November 2, 2020]. Available from: https://www.ucsfhealth.org/medical-tests/003359.
9. Okayama Y, Kuwahara M, Suzuki AK, Tsubone H. Role of reactive oxygen species on diesel exhaust particle-induced cytotoxicity in rat

cardiac myocytes. J Toxicol Environ Health A. 2006; 69(18):1699-710. doi: 10.1080/15287390600631078. PMID: 16864420.
10. Wihastuti TA, Sargowo D, Tjokroprawiro A, Permatasari N, Widodo MA, Soeharto S. Vasa vasorum anti-angiogenesis through H_2O_2, HIF-1α, NF-κB, and iNOS inhibition by mangosteen pericarp ethanolic extract (Garcinia mangostana Linn) in hypercholesterol-diet-given Rattus norvegicus Wistar strain. Vasc Health Risk Manag. 2014; 10: 523-31. doi: 10.2147/VHRM.S61736.
11. Forman HJ, Bernardo A, Davies KJ. What is the concentration of hydrogen peroxide in blood and plasma? Arch Biochem Biophys. 2016;603: 48-53.
12. Wihastuti TA, Aini FN, Tjahjono CT, Heriansyah T. Dietary Ethanolic Extract of Mangosteen pericarp Reduces VCAM-1, Perivascular Adipose Tissue and Aortic Intimal Medial Thickness in Hypercholesterolemic Rat Model. Open Access Maced J Med Sci. 2019;7(19): 3158-3163. doi: 10.3889/oamjms.2019.717. PMID: 31949509; PMCID: PMC6953934.
13. Satarug S, Vesey DA, Gobe GC. Kidney Cadmium Toxicity, Diabetes and High Blood Pressure: The Perfect Storm. Tohoku J Exp Med. 2017;241(1): 65-87. doi: 10.1620/tjem.241.65. PMID: 28132967.
14. Tremellen K. Oxidative stress and male infertility: A clinical perspective. Studies on Men's Health and Fertility 2012;14(3): 325–53, Doi: 10.1007/978-1-61779-776-7_16.
15. Naka KS, de Cássia Dos Santos Mendes L, de Queiroz TKL, Costa BNS, de Jesus IM, de Magalhães Câmara V, de Oliveira Lima M. A comparative study of cadmium levels in blood from exposed populations in an industrial area of the Amazon, Brazil. Sci Total Environ. 2020;698:134309. doi: 10.1016/j.scitotenv.2019.134309. Epub 2019 Sep 5. PMID: 31783457.
16. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. Journal of Environmental Pathology, Toxicology and Oncology: Official Organ of the International Society for Environmental Toxicology and Cancer 2000; 19(3): 201—213.
17. Xu X, Liao W, Lin Y, Dai Y, Shi Z, Huo X. Blood concentrations of Lead, Cadmium, mercury and their association with biomarkers of DNA oxidative damage in preschool children living in an e-waste recycling area. Environmental Geochemistry and Health. 2018;40(4): 1481–94, Doi: 10.1007/s10653-017-9997-3.
18. Nazimabashir, Manoharan V, Miltonprabu S. Cadmium induced cardiac oxidative stress in rats and its attenuation by GSP through the activation of Nrf2 signaling pathway. Chem Biol Interact. 2015;242: 179-93. doi: 10.1016/j.cbi.2015.10.005. Epub 2015 Oct 14. PMID: 26462792.
19. Eum KD, Lee MS, Paek D. Cadmium in blood and hypertension. Sci Total Environ. 2008;407(1):147-53. doi: 10.1016/j.scitotenv.2008.08.037. Epub 2008 Oct 8. PMID: 18845316.
20. Rafati RM, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 2017;8(3): 135-145.
21. Safa WA. Study of Heavy Metals and their effects on Oxidant / Antioxidant Status in Workers of fuel Station in Hilla city-Iraq. Research Journal of Pharmacy 2018;11(1): 312–6.
22. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease--a systematic review. Environ Health Perspect. 2007;115(3): 472-82. doi: 10.1289/ehp.9785. Epub 2006 Dec 22. PMID: 17431501; PMCID: PMC1849948.
23. Nigra AE, Ruiz-Hernandez A, Redon J, Navas-Acien A, Tellez-Plaza M. Environmental Metals and Cardiovascular Disease in Adults: A Systematic Review Beyond Lead and Cadmium. Curr Environ Health Rep. 2016;3(4): 416-433.
24. Amos MM, Carolina N, Taylor ML. Exposure to Lead, Arsenic, and Cadmium in an Inner City Neighborhood. Raleigh; North Carolina: 2017.
25. Eom SY, Lee YS, Lee SG, Seo MN, Choi BS, Kim YD, et al. Lead, Mercury, and Cadmium Exposure in the Korean General Population. J Korean Med Sci. 2018;33(2):e9.
26. Cook J. Environmental pollution by heavy metals. International Journal of Environmental Studies 1977;10(3): 253-266. doi: 10.1080/00207237708737323
27. Mohammed T, Mohammed E, Bascombe S. The evaluation of total mercury and arsenic in skin bleaching creams commonly used in Trinidad and Tobago and their potential risk to the people of the Caribbean. J Public Health Res. 2017;6(3).