Effect of Cadmium Exposure in Polymorphisms Gene NOS3, Blood Cadmium Level, Nitric Oxide Level, Blood Pressure and Antioxidant Enzymes

Hernayanti1*, Slamet Santoso1, and Sri Lestari1

1Biology Faculty, Universitas Jenderal Soedirman, Purwokerto - Indonesia

Abstract. Cadmium is one of a heavy metal which widely used in human life, especially in the electroplating industry and a mixture of textile materials. Cadmium that enters the body binds to the metallothioneins protein. It can increase the formation of free radical compounds, there by inhibiting enzyme activity such as nitric oxide synthase3. This gene regulates the expression of endothelial nitric oxide synthase which produce a nitric oxide. Nitric oxide role in regulated blood pressure as vasodilator with Angiotensin II as vasoconstriction. The susceptibility to Cd exposure will elevate if the polymorphisms of gene is found in population. The aim of this research was to know effect of cadmium to gene NOS3 polymorphisms on NO, systolic and diastolic blood pressure and antioxidant enzyme in Cd-exposed individual. The genotype individual were detected by Polymerase Chain Reaction-Restiction Fragment Length Polymorphisms (PCR-RLFP) with MBo1 restriction enzyme. Parameter recorded were blood Cd, NO level, SOD, systolic and diastolic. Data were analyzed by independent t-test. These result showed that 20% of 40 individual of cases subject were detected as polymorphisms individual of NOS3gene, with GA genotype. Their fragment DNA located on 206 bp, 119 bp and 87 bp, but non polymorphisms of NO gene is only located on 206 bp. The result show cadmium could influence polymorphisms NOS3gene and decrease NO production followed by increasing of blood pressure both systolic and diastolic. Cadmium also decrease antioxidant enzyme SOD and GPx level.

Keywords: Cadmium; systolic; diastolic; SOD; GPx

1. Background

Cadmium is a heavy metal which is belongs to class II B in the periodic system. This metal will readily react with ligands containing elements of O, S and N. In the body Cadmium is toxic agent, because it reacts with ligands that are essential for normal body function. In living cells there are various ligands, such as: OH, - COO-, -OPO3H-, -C = O, -SH, -S-S-, -NH2 and NH which can form complex bonds with metals such as Cadmium. Cd exposure also causes increased free radicals in the body and causes oxidative stress characterized by decreased levels of Super Oxide dismutase (SOD) and Glutathione peroxidase (GPx) [1-2]. Cadmium in the body accumulates in the kidneys and the liver mainly binds to metallothionein. The interaction between Cd and metallothionein, will inhibit enzymes activity and one of them is endothelial enzyme Nitric Oxide Synthase (eNOS) [3]. The enzyme eNOS is an enzyme that produces nitric oxide (NO) which functions in the regulation of blood pressure. The inhibition of eNOS activity cause the production of Nitric Oxide decreases result in decreasing elasticity of blood vessels. This will lead to increased vascular angiotensin II activity resulting in continuous vasoconstriction of blood vessels. This event causes elevated blood pressure called hypertension [4,5]. Association of blood cadmium with hypertension found in the Korean general population [6,5].

The susceptibility of exposure heavy metals in human will increase when gene polymorphisms are present [7]. Chuang et al. (2006) states that gene polymorphism is an important factor affecting human susceptibility to the toxic substances such as Cd. Polymorphisms at DNA level can cause decrease in enzyme activity by 20-30%. Nitric oxide synthase3 gene polymorphism (G894T) is found in exon 7, which converts glutamate amino acid into asparagine at the position of amino acid 298 [8]. G894T polymorphism causes eNOS enzyme activity to decrease, resulting in reduced NO production. Hernayanti et al., 2012 found that NOS3 polymorphisms in carrier polymorphism NOS3 gene individuals had lower NO levels in individuals without NOS3 gene polymorphism. Low NO levels will activate the matrix of metalloproteinase-9 and pro-inflammatory cytokines (Tumor Necrosis Factor α) and increased Matrix Metalloprotein-9 (MMP-9) but decreased their inhibitor namely Tissue Inhibitor Metallo Proteinase (TIMP), so hypertension develops into atherosclerosis [9-10]. Welding workshop workers are one group of individuals who are at risk of exposure to Cd and are at risk of developing hypertension, especially for individuals...
carrying NOS3 gene polymorphism. Early detection of hypertensive events due to Cd exposure, especially on the carrier of NOS3 gene polymorphism, through NOS3 molecular analysis and NO, SOD and GPx levels, systolic and diastolic blood pressure can help prevent early prevention of hypertensive disease from atherosclerosis.

Research by early detection of cadmium pollution, especially from the welding process, one of the source of cadmium exposure will contribute to reduce carbon emissions in the community. In welding process in addition to the Cd particles, carbon monoxide (CO) gas is also produced. CO gas is one of the pollutants in the air resulting from incomplete carbon combustion in gasoline. This will cause carbon emissions in the air to increase. Reduction of carbon emissions due to Cd and CO in the air means supporting a low-carbon program in community development so that people live more prosperously.

1. Material and Method
   Research Design

   The research design used an observational analytical design with cross sectional approach [11]. The research was conducted from June to September 2016 and the research located in welding workshop Purwokerto and Sokaraja. Target population is welding labores, while the population is affordable in this study are workshop workers in Purwokerto and surrounding areas amounted to 40 people. Sample size due to population less than 100 people, then all eligible populations of inclusion and exclusion criteria were studied. Inclusion criteria: respondents who were willing to sign Informed Consent, were not taking hypertension drugs and exclusion criteria were respondents who were taking hypertension drugs and respondents who not present at the time of the research. The independent variable is a Cd exposure. The dependent variables are the change Cd level in blood as well as changes in systolic blood pressure and diastolic welding labores. The parameters observed were systolic and diastolic blood pressure, Cd blood level, NO level, SOD and GPx activities.

   Determination of Cd level

   Respondents were taken blood in the vein section of mediana cubiti with the syringe of 5 mL. 5 mL of blood is divided into 3 parts, 3 mL for DNA isolation, 2 mL for Cd and NO examination. Cadmium level detected using ACGIH method with AAS machine at wavelength 228.6 nm and strong currents of 3.5mA.

   Isolation of DNA

   Isolation of DNA are performed with Guanidin method. (Hingorani,1999). Erythrocyte in blood sampel is lysed using lysing buffer. Then placed in refrigerator for 20 minutes and centrifuse 750 g at 8000 rpm for 10 minutes. The sampel was preparation with Guanidin, SE buffer, chloroform, NaCl 1 M and isopropanol and stored at 40°C.

   Determination of polymorphism NOS3 gene

   Amplification gene with PCR (Hingorani et al., 1999). Forward primer : 5'-CAT GAG CGT CAG CCC CAG AAC-3' and Reverse primer : 5'-AGT CAA TTC TTT TGG TGC TCA C-3', result product located at 206 bp. PCR conditions include a 5-minute denaturation of 94°C, 35 cycles consisting of 94°C denaturation 1 minute, annealing 60°C 1 minute, elongation 72°C 1 minute and final elongation 72°C, 7 minutes. PCR product was digested by MboI endonuclease restriction enzyme. The results of the digestion were analyzed by electrophoresis using agarose gel and visualized with ethidium bromide under UV light. The electrophoresis result were: GG was not cut off, TT was cut into 2 fragments along 119 and 87 bp, GA into 3 fragments along 206 bp, 119 bp and 87 bp.

   Determination of Nitric Oxide level

   Determination of Nitric Oxide level by Griess Method [12]. Measured simple absorbance value at wavelength 520-560 nm with microplate reader. Created regression curve Y: NO standard and X: NO concentration. Made the correlation regression curve based on the concentration of standard solution.

   Measurement of blood pressure by digital tensimete requirement

   Installed this instrument approximately 2 cm below the left hand, then tightened. Th tensimeter is turned on by pressing the on button. After 2 minutes exit the numbe indicates systolic pressure (uppernumber) and diastolic pressure (bottom number). The Reading results are expressed with mmHg units.

   Determination of SOD activity using RanSOD [13]

   Sample of SOD read on spectrophotometer with wavelength 505 nm. Standard curve graphs are created to determine SOD activity and results are expressed in U/L.

   Determination of GPx activity

   Determination of GPx activity [14]. Sample were read on Elisa Reader with wavelength 450 nm. Standard curve graphs are created to determine GPx activity and results are expressed in pg / mL units.

   Data analysis

   The molecular analysis of NOS3 gene polymorphism was analyzed descriptively, after the digestion results were visualized with ethidium bromide under UV light. Meanwhile, to know the effect of NOS3 gene polymorphism on blood Cd, NO level, GPx and SOD activity using Chi square test.

2. Result and Discussion

   The results of NOS3 gene analysis of 40 case-subjects after digestion with MBo1 enzyme were found that 8 individuals with NOS3 polymorphism or about 20% NOS3 gene polymorphism with GA genotype, while 32 or 80% were found without polymorphism with GG genotype. Individual DNA bands with polymorphism are cut into 3 fragments.
NOS - nitrative stress characterized by decreasing compare 4 5 6 7 8 9 10 UC M characterized by statistical NOS3

The mechanism of hypertension to Cd peroxide. These free radicals NOS different gene methallotionein in the liver and function, compared to into H reaction polymorphism carrier is higher than non require ion SOD gene (Figure 1).

The result of statistical test of diastolic blood pressure on individual carrier of NOS3 gene polymorphism is significant different compare with non polymorphism individual (t count = 4.35 and t table α1% = 2.704). Individual systolic pressure of NOS3 polymorphism carrier is higher than non polymorphism individual that is 157.75 ± 3.42 mmHg, whereas non polymorphism individual is 139.69 ± 11.32 mmHg.

The result of statistical test of diastolic blood pressure also showed very significant difference between individual carrier polymorphism with non polymorphism individual (t count = 4.93 and t table = α1% = 2.704). Individual diastolic blood pressure of polymorphism carrier is higher that is 95.37 ± 7.89 mmHg while non polymorphism individual is 85.12 ± 4.17 mmHg. The mechanism of hypertension to Cd exposure is due to the cadmium that enters the body by bonding with the amino acid metallothionein (Mt) to form Cd + Mt (cadmium + metallothionein) complex. The Cd + Mt bond is stable and will inhibit the endothelial activity of nitric oxide synthase (eNOS). The inhibition of eNOS activity cause the production of Nitric Oxide decrease result in decreasing elasticity of blood vessels. This leads to increase vascular angiotensin II activity, resulting in continuously vasoconstriction of blood vessels. These events cause elevated blood pressure or hypertension (4-5) 2 times from normal will decrease NO levels by 5% and increase systolic blood pressure by 0.75 mmHg and diastolic by 1.077 mmHg [15].

Based on the grouping of hypertension category, the result of group normotension 3 people (7.5%), group of pre hypertension 9 people (22.5%), group of hypertension level 1 counted 22 people (55%) and group of hypertension level 2 counted 6 people (15 %). Individual carriers of NOS3 gene polymorphisms with grade 2 hypertension were 5 (20%) while those with hypertension level 1 were 3 (7.5%). In non polymorphism group, the result of group normotensi 3 people (7.5%), group of pre hypertension 9 people (22.5%), group of hypertension level 1 were 17 people (42.5%) and found no group of hypertension level 2.

The result of statistical tests of NO levels in individual carriers of NOS3 gene polymorphism differed significantly with individuals without polymorphism (t count = -5.28 and t table α1% = 2.704). Nitric oxide concentration in individual carrier of NOS3 gene polymorphism was lower than non polymorphism of 27.4 ± 1.76 μmol / L, whereas in non polymorphism individuals were 37.85 ± 5.39 μmol / L.

Cd exposure in individual carriers of NOS3 gene polymorphism is more severe hypertension than that of non-polymorphism individuals. This is in accordance with the opinion of Chuang et al., 2006 which states that a person’s susceptibility to exposure to toxic compounds will increase when a gene polymorphism is found, as it will cause a 20% -30% decrease in enzyme function, compared to individuals without gene polymorphism.

The result of statistical test of SOD level showed very significant difference between individual carrier of NOS3 gene polymorphism with non polymorphism individual (t count = 4.24 and t table α1% = 2.704). SOD levels in individual carriers of the NOS3 gene polymorphism were lower at 112.87 ± 4.72 U / L compared with non polymorphic individuals with SOD levels of 137.85 ± 5.39 U / L.

Cd bonds with metallothionein in the liver and kidneys increase the production of free radicals in the body such as superoxide anions, hydroxyl radicals and hydrogen peroxide. These free radicals will lead to lipid peroxidation reaction that characterized by the elevated of mallondialdehyde (MDA) level and cause oxidative stress characterized by decreasing activity of the antioxidant enzymes SOD, GPx and catalase [16].

The function of SOD is to capture O₂ and convert it to hydrogen peroxide (H₂O₂). But if O₂ formation is excessive, SOD are not able to neutralize O₂ and convert into H₂O₂. The SOD enzyme located in the cytoplasm and extracellular require ion co-

![Image](https://doi.org/10.1051/e3sconf/20187306006)
factors Cu and Zn, whereas SOD located in mitochondrial requires the Mn co-factor. Cadmium is antagonistic to the metal ion cofactors. Cadmium will substitute the Cu, Zn and Mn, on the other hand the function of these metals are to arrange SOD, so that the SOD enzymes in the cytoplasm, extracellular fluid and mitochondrial become inactive [17-18].

The result of statistical analysis for GPx activity showed a very significant difference between individual carrier of NOS3 gene polymorphism compared with non polymorphism individual (t count = 8.46 and t table α 1% = 2.704). GPx activity in individual carriers of NOS3 gene polymorphism was lower at 45.33 ± 3.57 μmol /L, whereas the individuals with polymorphisms were GPx activity were 92.62 ± 15.33 μmol /L.

Cadmium could substitute Selenium (Se) which is a cofactor for GPx activity, so the GPx enzyme becomes inactive. The GPx function is to continue the SOD function to convert H₂O₂ to H₂O and O₂. As a result there is accumulation of free radicals of O₂⁻ and H₂O₂. Cd bonds with methallotionein on the liver and kidneys will decrease glutathione levels that are substrates for GPx. Glutathione also functions in the body's antioxidant defenses. Lack of glutathione production will cause damage to the liver and kidneys getting worse and will increase the toxicity of Cd (Johri, et al, 2010).

3. Conclusion

This result show that the exposure of Cd influence in NOS3 gene. Polymorphism of NOS3 gene in welding labour was found 20% and in non polymorphism individual 80%. Cd level, systolic and diastolic blood pressure in individual with NOS3 gene polymorphism is higher. And they have NO, SOD and GPx levels are lower than that of non polymorphism individual. The incidence of hypertension in individual carriers of NOS3 gene polymorphism is found as many as 5 people (20%) with hypertension level 2, while 3 people (7.5%) with hypertension level 1. In non polymorphism individuals is found normotension 3 people (7.5%), pre hypertension 9 people (22.5%), hypertension level 1 as many as 17 people (42.5%) and hypertension level 2 is not found.

Acknowledgement

On this occasion, we thank the Ministry of Research, Technology and Higher Education through the Steering Committee of Research and Community Service Institute of the University of Jenderal Soedirman, for the Fundamental Funds Fund for Fiscal Year 2016 and Research of Institutional BLU Fund for Fiscal Year 2017, so this researchcan be solved.

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