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International Conference Tropical and Coastal Region Eco-Development

Semarang, August 11-13, 2014

Venue : Patra Jasa Hotel S

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Research and Community Services Institute, Diponegoro University is organizing an international conference and Coastal Region Eco-Development. The conference is intended to promote and disseminate all research of : **(A). Coastal Region Eco-Development** and **(B). Tropical Life Sciences**. The scope of Coastal Region Eco-Development encompasses publications with topics: Coastal engineering, Marine Products Processing, Marine Biotechnology, Coastal Management and Social Economics, Disaster management, Food Technology and any other relevant engineering subjects related to coastal eco-developments. While the scope of **Tropical Life Sciences** covers public health , psychology, tropical diseases, policy related to health and disease; pharmacological aspects; epidemiology, genetic studies, medical microbiology, food nutrition and health, microbiology.

Keynote Speakers

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	Dr. Maria Barbosa is Director of AlgaePark at Wageningen university and Research Centrum , Netherlands
	Prof. Katsuo Miyashita , Bio-functional Material Chemistry, Faculty of Fisheries Sciences, Hokkaido University, Japan
	Dr. Sootawat Benjakul , Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Thailand
	Prof. Jamaludin Jompa is Director of Research Center for Marine, Coast & Small Island at Hasanuddin University
	Prof. Taufiq-Yap Yun Hin , Founder and current Coordinator for the Centre of Excellence for Catalysis Science and Technology (PutraCAT), Universiti Putra Malaysia
	Wil M.V. Dolmans , Em. Professor of International Health (Radboud University, Nijmegen, The Netherlands)
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Aortic Elasticity Profile of Children Living in Area of Chronic Organophosphate Exposure: A Preliminary Study ☆

Anindita Soetadji ^a  , **Suhartono** ^b, Apoina Kartini ^b, Budiyo ^b, Galuh Hardaningsih ^a, Agustini Utari ^a 

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Abstract

Brebes is an agricultural village in north coastal area of Central Java that is exposed to organophosphate pesticides. Organophosphate could cause mechanical damage of artery such as increasing the stiffness and leading to arteriosclerosis in early life. Aortic (Ao) elasticity profiles of children who are living in those polluted area have never been studied. The aim of study was to determine Ao wall stiffness and distensibility in children living in Brebes. A cross sectional study was conducted in fifty students aged 9-12 years (mean 9.24 SD 0.69), male 30 and 20 female, who were living in Brebes. Ascending Ao distensibility and stiffness were obtained on M-mode using Logic E echocardiography. Mean value of three times measurement was presented and compared with standard for healthy children according to the age. Mean and median of Ao-distensibility were 98.7 (SD 55.08) and 90.8 (13.98-224.55) [normal 97.1 (SD 47.6); 85.7 (22.6-368.5)]; Ao-stiffness index were 2.2 (SD 0.55) and 2.1 (1.28-4.06) [1.18 (SD 0.57); 1.05 (0.24-3.69)] respectively. Ao distensibility of subjects showed 16% lower, 62% normal and 20% higher than the mean of standard value. Ao stiffness index of subjects was 16% normal and 84% higher than standard. After adjusted with BSA, 78% of subject's Ao distensibility/BSA was normal, 96% has higher Ao

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Aortic elasticity profile of children living in area of chronic organophosphate exposure: A preliminary study

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Abstract

Brebes is an agricultural village in north coastal area of Central Java that is exposed to organophosphate pesticides. Organophosphate could cause mechanical damage of artery such as increasing the stiffness and leading to arteriosclerosis in early life. Aortic (Ao) elasticity profiles of children who are living in those polluted area have never been studied. The aim of study was to determine Ao wall stiffness and distensibility in children living in Brebes. A cross sectional study was conducted in fifty students aged 9-12 years (mean 9.24 SD 0.69), male 30 and 20 female, who were living in Brebes. Ascending Ao distensibility and stiffness were obtained on M-mode using Logic E echocardiography. Mean value of three times measurement was presented and compared with standard for healthy children according to the age. Mean and median of Ao-distensibility were 98.7 (SD 55.08) and 90.8 (13.98-224.55) [normal 97.1 (SD 47.6); 85.7 (22.6-368.5)]; Ao-stiffness index were 2.2 (SD 0.55) and 2.1 (1.28-4.06) [1.18 (SD 0.57); 1.05 (0.24-3.69)] respectively. Ao distensibility of subjects showed 16% lower, 62% normal and 20% higher than the mean of standard value. Ao stiffness index of subjects was 16% normal and 84% higher than standard. After adjusted with BSA, 78% of subject's Ao distensibility/BSA was normal, 96% has higher Ao stiffness index/BSA than mean standard value. This study found higher Ao-stiffness index among children living in organophosphate exposure area, despite of normal Ao distensibility.

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Keywords: aortic stiffness index, aortic distensibility, organophosphate exposure

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1. Introduction

Pesticides are commonly used in shallot plantation in Brebes. The children who are living in this village are prone to pesticide exposure directly and indirectly through polluted environment. One type of pesticides that is widely used in Brebes is organophosphate. Pesticide exposure in Brebes has proved to affect health and found to be risk factor for subclinical hypothyroidism[1]. Organophosphate exposure also may induces lipid peroxidation[2] damages vascular endothelial function[3], alters nitric oxide (NO) production in vascular smooth muscle cells[4] and changes modulus elastic of aorta[5] Chronic pesticide exposure in children is a risk factor of stunting and low body mass index (BMI)[6,7]. Organophosphate exposure during prenatal period reduces insulin like growth factors-1 (IGF-1) and leads to arterial stiffening in children[8]. Stefanadis et al. found that the distensibility of ascending aorta that was measured noninvasively 30 mm in the distal of aortic cusp with echocardiography was closely related to angiographic invasive study[9]. Increased arterial stiffness in adolescents is associated with left ventricular mass index independently of traditional risk factors[10] Changes of arterial function including aortic distensibility and stiffness are the parameter of vascular damage that could be detected initially[11]. In adults, ascending aorta distensibility is the best marker of subclinical large artery stiffening[12] that has been shown to be an independent predictor of progressive aortic dilation and dissection[13]. Whether there are any changes of aortic elasticity among children who were living in Brebes has never been studied yet. The aim of study was to assess the Ao stiffness and distensibility of children who were living in agricultural area of Brebes that was chronically exposed to organophosphate pesticide.

2. Method

A cross sectional study was conducted in Brebes, a village along north coastal area of Central Java, which was suggested had been chronically exposed to pesticide. Period of study was in 2014. This study was approved by the Ethics Committee of Public Health Research, Faculty of Public Health, Diponegoro University.

2.1. Subjects

Subjects were children aged 9 – 12 years in Dukuhlo 1, Brebes elementary school. Written informed consent were obtained from parent of subjects. Subjects were underwent clinical examination of anthropometric parameter, physics, blood pressure and echocardiography study.

2.2. Anthropometry measurement

Body weight and height, body mass index (BMI) and body surface area (BSA) measurement was conducted by trained health worker. All of these examinations were done in the morning with all of the students were wearing sport suit without shoes. Digital SECA 874 was used for body weighing. The height was measured using *microtoir*. BMI and linear growth was determined using WHO anthropometric standard calculator.

2.3. Blood pressure examination and echocardiography study

Blood pressure and echocardiography study were performed simultaneously after subjects at rest for 10 minutes. Blood pressure examination was conducted using Omron digital blood pressure monitor. Transthoracic echocardiography study was performed using Logic E portable echocardiography device, while subject was lying in supine position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was obtained by physician on the left upper arm for three times at the same time. The mean of SBP and DBP were drawn for calculation. Blood pressure then classified according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents of U.S. Department of Health and Human Services[14].

Echocardiography study was performed by pediatric cardiologist using a Logic E portable (General Electric) echocardiography device. Complete study were performed to rule out cardiac structural and functional abnormality. Children with cardiac abnormality were excluded from study. Ascending aortic distensibility and stiffness study was

obtained with M-mode measurement in parasternal long axis view. Measurement took place at 30 mm distal of aortic valve. Inner to inner systolic and diastolic aortic vessel diameter were obtained three times for each subjects and stored for the availability for off-line calculation. Subjects were lying in supine position during examination. Aortic distensibility and stiffness calculation were calculated using formula as follows:

$$\text{Aortic distensibility} = (A_s - A_d) / [A_d \cdot (P_s - P_d) \cdot 1333] \cdot 10^7 \text{ (} 10^{-3} \text{ kPa}^{-1}\text{)}, \quad (1)$$

$$\text{Aortic stiffness index} = [\ln(P_s/P_d)] / [(D_s - D_d)/D_d] \text{ (dimensionless)}. \quad (2)$$

$$\text{Area (A) was determined as: } (A = (D/2)^2 \times \text{Pi}), \quad (3)$$

$$\text{Aortic strain} = S = (D_s - D_d) / D_d \quad (4)$$

where A is area, Ad is end-diastolic area; Ds, systolic diameter; Dd, diastolic diameter; Ps is SBP; Pd is DBP, both are in mmHg.

3. Result and Discussion

Fifty students (30 boys, 20 girls) were included in the study. The mean and median of age were 9.24 (SD 0.69) years and 9 years old (range 8 – 10 years) respectively. Anthropometric data was shown in table 1.

Table 1. Anthropometric characteristics of the subjects

	Mean (SD)	Median (range)
Body weight (kg)	26.2 (5.48)	25.1 (18.50-44.75)
Height (cm)	127.1 (6.18)	127.3 (116.30-140.35)
BMI	16.1 (2.11)	15.4 (12.91-24.21)

According to WHO anthropometric standard, 14/50 (28%) of subjects were short stature. Nutritional state according to the body mass index [BMI = weight/(height)²] WHO classification revealed 48/50 (96%) subjects were normal and only 2/50 (4%) were undernutrition. It showed that the subjects were chronically undernourished. No subjects who were obese.

Table 2. Blood pressure and heart rate measurement

	Mean (SD)	Median (range)
Systolic blood pressure (SBP) (mmHg)	93.6 (9.58)	92.7 (75.00-114.67)
Diastolic blood pressure (DBP) mmHg	57.7 (6.59)	57.7(44.67-72.67)
Heart rate (HR) beat/min	102.6 (11.41)	100.00(77-135)

Blood pressure was divided into hypotension, normal and hypertension according to standard normal value for children according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents of U.S. Department of Health and Human Services,[14] showed that 36/50 (72%) of subjects were hypotension, and the rest were normotension. No subjects had high blood pressure.

Table 3. Aortic stiffness and distensibility

	Mean (SD)	Median (range)
Ao systolic diameter (cm)	1.83 (0.171)	1.84 (1.42-2.21)
Ao diastolic diameter (cm)	1.52 (0.152)	1.53 (1.20-1.81)
Aortic distensibility	98.66 (55.09)	90.75 (13.98-224.55)
Aortic stiffness	2.21 (0.55)	2.13 (1.28-4.06)
Ao distensibility/BSA	104.05 (60.39)	89.06 (16.47-249.05)
Ao stiffness/BSA	2.35 (0.71)	2.27 (1.20-4.79)
Aortic strain	0.20 (0.09)	0.20 (0.03 – 0.45)

According to mean normal value of Ao stiffness index for appropriate age that has been studied previously by

Hauser M et al [15] that was 1.18 (SD 0.57); our study found that 42/50 (84%) of subjects had higher mean Ao stiffness index, while only 8/50 (6%) were within normal value. After adjusted to body surface area (BSA), 48/50 (96%) of subjects had higher Ao stiffness index/BSA than normal value that found by Hauser M et al [mean normal value for Ao-stiffness index/BSA 0,89 (SD 0,37)]. Ao distensibility value showed that 39/50 (78%) of subject were within normal limit, 10/50 (20%) were higher and 1/50 (2%) were lower than normal limit.

Organophosphate exposure had been known could damage mechanical properties of rat's aorta.[16] There is not only vascular structure that could be damaged by chronic pesticide exposure, but also other organs including liver cells [2], autonomic nervous system[17], and decreases IGF-1 level [8]. IGF-1 is important for endothelial cells recovery from damage, NO production[18,19] and regulation of glutathion peroxidase expression and activity[20].

Our data demonstrated that the mean and median of aortic stiffness index of subjects were higher than healthy children in the similar age that have been studied by Hauser et al (2013) [mean 2.21 (SD 0.55) vs 1.18 (SD 0.57); median 2.13 (1.28-4.06) vs 1.05 (0.24-3.69)] while the aorta distensibility was within normal limit [mean 98.66 [SD 55.09] vs 97.1 (SD 47.6); median 90.75 (13.98-224.55) vs 85,7 (22,6-368.5)]. Subjects in Hauser's study were similar in the range of age, but different in ethnicity and BMI. Subjects's BMI in our study were smaller than Hauser's [16.1 (SD 2.11) vs 18.3 (SD 2,9)]. There were also difference of heart rate, which the heart rate of our study was faster than Hauser's. According to Hauser's study finding that stiffness increases concordance to BMI, we anticipated that the result of our study would show lower aortic stiffness index rather than higher. Even after adjusted with BSA, the result of our study shows higher aortic stiffness index [2.35 (SD 0.71) vs 0.89 (SD 0.37)].

Present data indicates that there is health problem that altering aorta elasticity of children in Brebes. Normally, Ao stiffness index would be increasing, whilst the distensibility would be decreasing with aging[15]. Increase Ao stiffness in long-term period would increase left ventricular mass and leading to left ventricular diastolic dysfunction of the heart[21].

Tonus of artery is regulated by NO production that is induced by endothelial NO synthase (eNOS) in endothelial cells. NO that produced by endothelial cells will diffuse in to vascular cell muscle cells promotes synthesis of 3,5-cyclic guanosine monophosphate that leading to vasorelaxation[22]. Major stimulus of eNOS production is shear stress that physiologically increases during activity and other condition that rises heart rate and cardiac output. Several factors that have been known could damage endothelial artery and leading to dysfunction are oxidative stress and hypercholesterolemia[23], diet and exercise[24] obesity[25,26,27], and hypothyroidism[28]. Pesticide exposure also reduces growth during peri-pubertal period [7] Chronic antenatal exposure could reduce birth weight and growth hormone-IGF-1 system, that leading to cardiovascular profile changes at the school age.[8]

IGF-1 have important role on cell recovery of damaging endothelial cells layer. Endothelial artery protection by IGF-1 occur through endothelial and vascular smooth muscle cell growth stimulation and NO production that induced by endothelial NO synthase (eNOS) pathways.[18] IGF-1 that circulates in the blood was produced in the liver under stimulation of growth hormone. IGF-1 that is produced by liver then will be released in circulation and bind to IGF-1 binding protein (IGFBP) and circulate to target organs[29]. IGF-1 is also produced locally, including growth plate. IGF-1 deficiency in children is associating with linear growth in children, leading to short stature. Heart and vasculature are important IGF-1 target organs, which IGF-1 promotes upregulation of antioxidant enzymes and exerting mitochondrial protective effects. IGF-1 also promotes progenitor cell function, improves NO bioavailability, and limits apoptotic cell death. Circulating IGF-1 level decreased with aging[30].

Our study found that 28% of subjects were short stature. What exactly the etiology of short stature in this subjects is beyond of present study. Short stature is a common terminology for height for age below normal standard. Several factors that cause short stature are nutritional insufficiency, congenital, constitutional, familial, and endocrine causes. In nutritional insufficiency, the weight declines before the length and the weight for height is low, unless there has been chronic stunting. In congenital pathologic short stature, an infant is born small and growth gradually tapers off throughout infancy. The causes of congenital pathologic short stature are include chromosomal abnormalities (i.e. Turner syndrome, trisomy 21), infection (i.e. TORCH [toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex] infections), teratogens drugs (i.e phenytoin [Dilantin], alcohol), and condition of extreme prematurity. Constitutional growth delay signs are weight and height that decrease near the end of infancy, parallel the norm through middle childhood, and then accelerate toward the end of adolescence, adult size is normal. In familial short stature, both the infant and parents are small; growth runs parallel to and just below the normal curves[31]. Chronic malnutrition could leading to micronutrient deficiency and caused stunting[32]. However, there is possibility that high prevalence of stunting in Brebes is correlated with oxidative stress by pesticide exposure that may affect the appetite, and decreasing IGF-1 level thus compromising linear growth.

According to IGF-1 protection on vascular function, it will be interesting to study whether higher aortic stiffness in children who are living in Brebes is correlated with decrease linear growth. This may reflect pathological conditions caused by oxidative stress and low IGF-1 level, due to chronic pesticide exposure. This allegation warrants further study.

Noteworthy, the system of portable Logic E equipment using in this study is unable to detect aortic wall automatically, thus the tip of inner-to-inner aortic wall should be recognized and marked carefully. The examination was also obtained by single experienced examiner who blinded to subjects and the measurement were taken three times, in order to reduce measurement error. The blood pressure examination was taken three times not continuously monitored during study. Despite of this limitation, those equipments are available in Indonesia and practically use for community study according to amenity and possibility for longterm follow up in this population.

4. Conclusion

Ascending aorta of children who are living in Brebes is stiffer than normal. Further study is required to find out whether it is caused by pesticide exposure or other risk factors and what is the correlation with the high prevalence of stunting in this population.

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