RESEARCH ARTICLE

Biomonitoring of Acetylcholinesterase (AChE) Inhibitor and the Association with Hypertension among Farmers in Bandung, Indonesia

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Abstract

BACKGROUND: The use of acetylcholinesterase (AChE) insecticides is still widely used by farmers in flower and agricultural centers. However, biological monitoring of farmers is still very rare in Indonesia. AChE inhibitors are reported to have toxic effects on various organs.

METHODS: This study involved 120 subjects in Cihideung, Cikole and Pangalengan areas. All subjects have been interviewed, physically examined and biological sample taken by medical team. Descriptive analysis was performed to assess general conditions of the subjects and AChE erythrocyte activity enzyme at pseudo-baseline and the next 3 months from pseudo-baseline. Statistical analysis have been performed of the pseudo-baseline AChE erythrocyte activity with hypertension and history of exposures.

RESULTS: The median value of pseudo-baseline AChE erythrocyte activity was 8.10 (1.3-14.25) U/g hematocrit. In the comparison between pseudo-baseline and 3 month from pseudo-baseline AChE activity, 7 respondents from 19 respondents (36.84%) had lower enzyme activity than 70% and the others subjects have higher activity value. AChE erythrocyte activity is associate with frequency of insecticide exposures. AChE erythrocyte activity (p=0.04; Exp (B)=2.937 CI 95%=1.049-8.224) and age (p=0.025; Exp (B)=3.872 CI 95%=1.180-12.703) are independent risk factors for hypertension in farmworker.

CONCLUSION: AChE erythrocyte activity associated with frequency of insecticide exposures and hypertension among farmworkers.

KEYWORDS: AChE erythrocyte activity, frequency of insecticide expsoures, hypertension

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Introduction

Pesticides are any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit.(1) Pesticides were classified in to many groups; insecticides, herbicides, rodenticides, fungicides, molluscicides, nematicides and others. Insectides are used intensively in agriculture to control insects.(2) Organophosphorous and carbamates insecticides are still used intensively in agricultural to control insects.

Chemically, there are two types of acetylcholinesterase (AChE) inhibitor insecticide; organophosphate and carbamates. Carbamates are class of insecticides with similar structure and mechanism of action to organophosphates insecticides. Carbamates are derived from carbamic acid and cause carbamylation of AChE at neuronal synapses and neuromuscular junctions.(3) Organophosphates are the most potent AChE inhibitor insecticide.(4) Signs and symptoms of organophosphate poisoning can be divided into central and pheripheral nervous system effects. Organophosphate insecticide poisoning can be acute or chronic. The symptom onset is categorized as acute (within 24 h), delayed (24 h to 2 week) and late (beyond 2 week).(5) The severity of organophosphate depends on pesticide type, dose, duration and frequency of application.

Airway exposure is a major risk for AChE inhibitor insecticide poisoning.(6) Some symptoms of acute poisoning are seizure, bradicardia, hypersecretion, bronchoconstriction, cyanosis, bradycardia, depression in the central nervous system, hypersecretion, myosis, cyanosis, diarrhea and coma. Acute symptoms will appear within 24 h after contact with the insecticide.(7-10) The chronic organophosphate insecticide poisoning was characterized by distal weakness that occurs 2-4 weeks after exposure. The symptom of chronic poisoning was cramping pain, weakness, fasciculations, twitching and paresthesias of the extremities followed by weakness of the distal limb muscles, especially in the legs.(5,11) Since symptom onset and mechanism of delayed manifestations (*e.g.*, intermediate syndrome, delayed onset coma that typically occur within 2 weeks) are dissimilar to late manifestations (*e.g.*, organophosphate induced delayed polyneuropathy that typically occurs after 2-3 weeks).

Spraying process are the most risk factor for insecticides inhalation. In spraying process, they spend 3 - 6 h per day. Wind direction, properly masker usage, spraying body position, insecticides dose, duration and frequency of spraying process may influence insecticides absorption during spraying process. Foods and water consumption may contaminated by insecticides deposition or residue.(12)

Organophosphate insecticide is the most potent AChE inhibitor class. It was known two types of AchE, true AChE and pseudo-AChE (BChE). True AChE is found in erythrocytes, nerve cells and skeletal muscle. Pseudo AChE is found in plasma and liver cells.(13,14) AChE erythrocyte was used as biomarker in occupational setting exposed chronically AChE inhibitor, but its very difficult to practice in the field. AChE erythrocyte test need baseline enzyme activity to compare with 3 months from pseudo-baseline activity. In the most agricultural field is very difficult to obtain the baseline activity because most of farmers were exposed for long time ago. The spray inseticide every 1 or two times per week.

There were two objectives of this study. Firstly, monitoring of AChE erythrocyte activity value in farmworkers, the change between pseudo-baseline and 3th month from the pseudo-baselin, and the association between AChE erythrocyte activity and some risk factors among farmworkers. The 3 months from the pseudo-baseline condition refers to 3 months after first sampling and subject exposed to AChE inhibitors in the field or working area. Secondly, the relationship between clinical conditions or symptoms with AChE activity and some risk factors for clinical conditions. The result was expected to provide information on the relationship of clinical conditions, especially hypertension, with AChE activity.

Methods

Study Location and Population

In this study, we examined the AChE activity from farmworkers that used inhibitor AChE insecticide from farmworker living in 3 villages located in the North and South Bandung, West Java, Indonesia. These area have been surveyed that the farmers used with various types of insecticides including AChE inhibitors, synthetic pyrethroid, mancozeb, abamectin, and imidacloprid.

The first study village (A) was located in the southern foothills of Mt. Tangkuban Parahu, 18.1 km from central Bandung. Most of resident of the village area consisted of flower fields and some agricultural fields. The most of resident used organophosphate insecticides and mixed with fungicide and abamectin. The second study village (B) was located in the southeast foothills of Mt. Tangkuban Parahu, 16 km from central Bandung. Most of resident of the village area consisted of vegetables crops field. The most of resident used organophosphate insecticides and mixed with fungicide, synthetis pyrethroids and abamectin. The third study village (C) was located between Mt Patuha, Mt Malabar and Mt Kendang, 44.6 km from central Bandung. Most of resident of the village area consisted of vegetables crops field. The most of resident used imidaclopride as insecticides, fungicides and, synthetic pyrethroid. In this village, organophosphate and carbamate insecticide usage are limited.

Study Design

A cross-sectional cohort design was used in this study. The first and the second sampling periode have been completed at March and July 2019, respectively. During sampling; antrophometric measurement, health examination and questionnaire were conducted for all subjects. As much as 130 subjects were recruited and 10 subjects were excluded. Inclusion criteria in this study was male, aged more than 20 years old, still active as farmworkers and more than one years as farmworker. All subjects spray AChE inhibitor 1 or more times per week, routinely. The exclusion of this study was severe anemia, malaria, antimalaria therapy and blood cancers. Before the examination, subjects were given information the benefits and risks of this study, interviews, study steps and the informed consent forms.

Ethical Clearance

The ethical protocol was evaluated and approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia with reference No. 1353/UN2.F1/ETIK/2018. All subjects who agreed to participate in this study were required to sign informed consent.

Biological Monitoring Sampling

Blood samples were taken from veins using vacuum containers with ethylenediaminetetraacetic acid (EDTA) anticoagulants after the working hour. Plasma and erythrocytes must be separated within 8 hours of sampling. Hemolysis must be avoided. Erythrocytes were washed with physiological saline solution. After erythrocyte isolation, the erythrocytes were haemolized by ultrapure water quantitatively. The haemolysates were sent immediately to the laboratory for AChE erythrocyte activity determination. The temperature was kept below 8^{o}C during transportation. The duration of sample transportation from the sampling locations to the testing laboratory was not more than 5 hours. The stability of this enzyme in the haemolized sample below 8^{o}C is one week without significant activity change.

AChE erytrocyte activity measurement

The method has been evaluated in Prodia IndTox Lab with the precision value between 3.30-9.42%. In the method reference, the precision value was <12.5%.(15) AChE erytrocyte was determined in Prodia Industrial Toxicology, Cikarang, West Java, Indonesia. AChE activity was determined photometrically by means of a kinetic test. After isolating, washing and hemolysing, the RBCs were mixed with the substrate acetylthiocholine iodide to determine the AChE activity. Acetylthiocholine iodide was converted to thiocholine iodide and acetate by the corresponding esterase. The reaction between Thiocholine iodide and 5,5'-dithio-bis-2-nitrobenzoic acid will induce yellow dye-stuff 5-thio-2-nitrobenzoic acid. The rate of dye formation occurs proportionally to the enzyme activity. The extinction coefficient was measured 4 times at a maximum wavelength (405 nm) after addition of the substrate. The mean of the 2nd to 4th extinction coefficients was calculated. Enzyme activity was obtained from the difference between the mean extinction coefficient and the first extinction coefficient.

Hematocrit Measurement

Hematocrit was used to compensate the volume of erythrocytes that used for AChE activity measurement. Hematocrit was measured indirectly through the average size and number of erythrocytes. Coulter Impedance principle in the reference of hematocrit measurement. Hematocrit was measured in Prodia Occupational Health Clinic, Cikarang, West Java Indonesia, using Abacus 380 hematology analyzer (Diatron, Jakarta, Indonesia).

Blood Pressure Measurement

Systolic and diastolic blood pressure were measured with calibrated sphygmomanometer, in the right arm, following protocols recommended by the American Heart Association.(6) Blood pressure measurements were taken after 10 min of rest. The subject was in a sitting position with antecubital fossa as high as the heart, with uncrossed legs. Blood pressure was measured twice, and the average systolic and diastolic blood pressure was used in the analysis. Hypertension criteria was referred to Join National Committee (JNC) 7.(16)

Statistical Analysis

Statistical analysis of data was conducted using SPSS 11.5 computer software (IBM Coorporation, Armonk, New York, USA). Firstly, descriptive analysis (median, minimum and maximum values) were performed as the characteristics for the study population. The AChE activity between pseudobaseline and 3 months from the pseudo-baseline was performed as percentage value. The relationship between AChE erythrocyte activity and work safety, insecticides exposure and job experiences were analyzed using Spearman's rho test.

Secondly, the relationship between AChE activity and clinical findings or symptoms were analyzed with Spearman's rho test. The receiver operating characteristic (ROC) was used to test between AChE activity and significant clinical finding variables. ROC analysis is used to obtain the cut-off value for AChE enzyme activity. Furthermore, multivariate analysis was conducted between significant clinical finding variable, AChE activity and other variables to determine the most powerful risk factors for clinical condition finding.

Results

Characteristic of respondents, the change of AChE activity and the relationship between AChE erythrocyte activity and work safety, insecticides exposure and job experiences.

Characteristics of the Respondents

This study involved 120 respondents from Cihideung (67 subjects), Cikole (19 subjects) and from Pangalengan (34 subjects) Bandung and West Bandung county regions in West Java. The median age of respondents farming population was found at 43 years old between 20 and 81 years old. All of respondents are male. Working period at the same jobs between 1 year up to 59 years with median value at 15 years. Education history of the respondents mostly were 50% has low education, 47% has middle education and only 2.5% has higher education. Median value of AChE activity was 3,671.49 U/L and between 512-6,882 U/L. In the corrected AChE activity with hematocrit was between 1.30-14.25 U/g hematocrit and the median value was 8.10 U/g hematocrit. The characteristic of respondents were presented in Table 1.

Table 1. Demographic characteristics of the study population.

Parameters	Median	Min-Max
Age (years)	43	20-81
Working period (years)	15	1-59
Pseudo-baseline AChE activity (U/g hematocrit)	8.1	1.3-14.25
Cihideung	7.67	1.3-13.92
Cikole	8.59	2.59-14.25
Pangalengan	8.2	5.64-11.35

The Change of AChE Activity

There were only 19 respondents out of 120 respondents who took second sampling (15.83%). The level of compliance and awareness of farmers to health was still very low. If pseudo-baseline activity was compared with 3 months from pseudo-baseline, 7 respondents from 19 respondents (36.84%) had AChE erythrocyte activity lower than 70%. Based on American Conference of Governmental Industrial Hygienists (ACGIH) (17) and MAK (15) biomonitoring guidelines, AChE erythrocyte should higher than >70% from the baseline. The change of AChE activity were presented in Table 2.

Relationship between AChE Erythrocyte Activity and Work Safety, Insecticides Exposure and Job Experiences

The number of subjects in the second sampling (3 months from pseudo-baseline) was small. The analysis of the relationship could not be conducted between the difference in AChE activity with safe work risk factors, history of exposure and work experience. Nevertheless, the analysis was still carried out between pseudo-baseline enzyme activity and these risk factors. The results were obtained that pesticide powder mixing, seeds planting and total pesticide exposure scores were associated to erythrocyte

Table 2. The change of AChE activity.

Respondent Coding	AChE Pseudo-baseline Activity (U/g hematocrite)	AChE 3 Months from the Pseudo-baseline Activity (U/g hematocrite)	AChE Erythrocyte Activity Change (%)
A	6.95	8.16	117.4
B	8.88	7.01	79.03
C	11.73	4.78	40.79
D	13.76	8.14	59.14
E	4.76	4.1	86.11
F	3.23	10.84	335.94
G	2.59	6.18	238.75
H	5.11	6.11	119.46
I	10.81	7.19	66.49
J	8.58	8.46	98.6
K	5.71	7.36	128.82
L	4.87	6.69	137.47
M	2.7	4.63	171.39
N	6.01	5.18	86.2
O	14.25	7.05	49.5
P	23.22	6.15	26.63
Q	6.12	5.88	96.07
R	8.6	4.63	53.91
S	6.7	4.57	68.16

Table 3. The relationship between AChE erythrocyte activity and work safety, insecticides exposure and job experiences.

Risk Factors	Rho Spearman	*p*-value*
Work safety		
Use of personal protective equipment	0.170	0.069
Hand cleansing	0.015	0.875
Change clothes immediately	0.040	0.475
Total score work safety	0.155	0.092
Frequency of insecticide exposures		
Duration of spraying	-0.060	0.527
Pesticide powder mixing	**0.193**	**0.039**
Seeds planting	**0.181**	**0.053**
Fertilizing	0.150	0.109
Cleaning of spraying equipment	0.060	0.527
Frequency of insecticide use total score	**0.184**	**0.046**
Other activity in the workplace		
Eating in the spraying area	-0.097	0.295
Drinking in the spraying area	-0.104	0.261
Smoking in the spraying area	-0.171	0.064
Working period in the spraying area	-0.007	0.944
Total score of job experience	0.040	0.670

*Spearman correlation test.

AChE activity. Safe work history and other activity in the workplace have no association with AChE erythrocyte activity. The association between AChE erythrocyte activity and occupational dan other activity in the workplace were presented in Table 3.

The Relationship between Clinical Finding and AChE Erythrocyte Activity

Some symptoms were find while sampling time. These symptoms were analysed with AChE erythrocyte activity using Spearman Rho test. Headache, convulsion, limb weakness and tremor were not associate with AChE erythrocyte activity. Hypertension was associate with AChE erythrocyte activity. The high blood pressure was associate with low of AChE erythrocyte activity (r=-0.212; p= 0.020). These findings were presented in Table 4.

Table 4. The relationship between clinical finding and AChE erythrocyte activity.

Clinical Finding	Rho Spearman	*p*-value*
Headache	-0.092	0.751
Convulsion	-0.009	0.919
Limb weakness	0.029	0.755
Tremor	-0.156	0.09
Hypertension	**-0.212**	**0.02**

*Spearman correlation test.

An receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off point with the specificity and sensitivity value of ACHe activity with hypertension. The result shows that the AChE erythrocyte activity at 6.2 U/g hematocrit has sensitivity level of 78% and specificity of 52%. ROC curve of AChE erythrocyte activity and hypertesion was presented in Figure 1.

Multivariate analysis has been conducted between erythrocyte AChE activity, other risk factors and

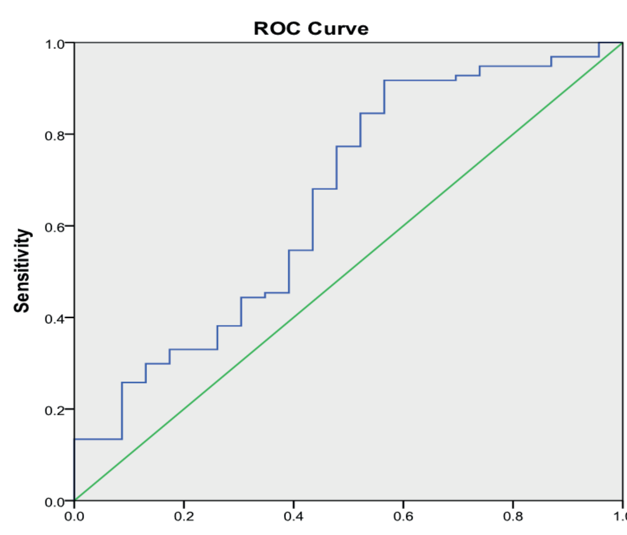

Figure 1. ROC curve of AChE erythrocyte activity and hypertesion.

hypertension. The result show that erythrocyte AChE (p=0.04; Exp (B)=2.937 CI 95%=1.049-8.224) and age (p=0.025; Exp (B)=3.872 CI 95%=1.180-12.703) are risk factors for hypertension. Working location, working period and central obesity were not associated with hypertension (p>0.05). Data from multivariate analysis of risk factors for hypertension and AChE erythrocytes are described in the following Table 5.

Discussion

Until now organophosphate insecticides are still widely used among farmers to reduce insects attacks. Previous studies showed that occupational exposure to organophosphate and carbamete induce acetylcholinesterase (AChE) activity reduction. These studies have been reported in Nepal (18), Tanzania (19), Australia (20) and Mexico (21). In our study, biomonitoring of erythrocyte AChE activity was carried out in several agri-horticultural locations in Bandung. Bandung is one of the centers of agriculture in Indonesia. Erythrocyte AChE activity at initial measurement has a median value of 8.10 U/g hematocrit with a minimum - maximum value of 1.3-14.25 U/g hematocrit. Respondents with low erythrocyte AChE activity were found in Cihideung and Cikole areas. Cihideung was a center for flower cultivation while Cikole was center for agricultural plants. This finding was consistent with the of organophosphate insecticides spraying frequency and dose in these areas.

The percentage of monitoring after 3 months from the pseudo-baseline was very low (15.83%). The health awareness and level of compliance of farmworkers were very low. The main reason for not taking part in the second sampling was that had long worked and lived in an agricultural area without significant health complaints. Another reason is the fear of blood redrawn. The initial AChE activity becomes a baseline (pseudo-baseline) data that will be used as a baseline for 3 months from pseudo-baseline AChE measurement. The limitation of this pseudo-baseline was exposed enzyme activity. In the field setting was very difficult to get un-exposed AChE enzyme. All of subjects used insecticides including AChE inhibitor class 1 or more than per week. In agriculture field there was almost impossible to stop the spraying process because it will inhibit and reduce agricultural products. This situation will affect the economic conditions of the subject. We found that 7 from 19 subjects (36.84%) have decreased AChE erythrocyte enzyme activity compare to the pseudo-baseline activity level. This finding could not to be analyzed with risk factors or clinical findings.

Exposure analysis was performed on pseudo-baseline AChE activity data. The results show that exposure factors have significant role in the erythrocyte AChE activity. The AChE activity level was very dynamic depend on frequency and duration of exposure risks between subjects. These exposure risk factors were mixing pesticide powder, planting seeds and total exposure history score. These risk factors can occur during the pouring, stirring and mixing of pesticide powder. The seed planting could be risk factor for insecticide exposure. In this task, insecticide particles could be dispersed into the air and could be inhaled through the respiratory tract. These finding was consistent with previous study.(10,22,23)

Biomonitoring guidelines for AChE inhibitors carried out against the pseudo-baseline and 3 months from the pseudo-baseline are difficult in the field practice. This study assesed the relationship between erythrocyte AChE activity and clinical disorders related-AChE inhibitor toxicity. Some clinical findings or symptoms (headaches, convulsions, muscle weakness, tremors and hypertension) were found during the study. After statistical analysis, hypertension has significant relationship with AChE activity. Hypertension and other cardiovascular disorders have been associated to organophosphate insecticides exposure.(24-28) ROC analysis was carried out to assess the sensitivity and specificity of AChE activity with hypertension. The results show that the AChE erythrocyte activity at 6.2 U/g hematocrit has sensitivity level at 78% and specificity level at 52% as in Figure 1.

Multivariate analysis of hypertension was carried out on AChE activity (at 6.2 U/g hematocrit) with other risk factors for hypertension (obesity, age, working period and

Tabel 5. Multivariate analysis between hypertension, AChE activity and others factors.

Variables	B	Wald	*p*-value	Exp(B)	CI 95%
Location	1.747	3.249	0.071	5.737	0.859-38.338
Working period	0.337	0.774	0.379	1.4	0.661-2.964
Central obesity	0.045	0.027	0.87	0.956	0.662-2.989
Age (years)	1.354	4.987	0.025	3.872	1.180-12.703
AChE activity (U/L)	1.077	4.204	0.04	2.937	1.049-8.224

working location). There was found that AChE erythrocyte activity and age more than 40 years were independent predictors of hypertension. Obesity in this study was not related to hypertension because the number of obese subjects were very small.

In chronic pathophysiological AChE toxicity, AChE inhibitor will inhibit AChE activity and acetylcholine will stimulate the post-synaptic neuron receptors continuously.(29-31) Acetylkoline will bind to the muscarinic and/or nicotinic receptors. Nicotinic N2 (Nn) receptors that act on the autonomic ganglion and adrenal medulla. Clinically, they will induce tachycardia and hypertension.(5,32-34) AChE inhibitor which have high affinity with AChE or have high binding specificity with nicotinic N2 (Nn) receptors will induce hypertension or tachycardia. The following are the types of organophosphate insecticides that are widely used by farmers in this study, including chlorpyrifos, profenofos, phenthoate. No association was found between headache, tremors, convulsions, muscle weakness and AChE activity.

This study needs further confirmation of the cut-off point value, for example by measuring other health problems induced-AChE exposure insecticides. The subject of this research is not only using organophosphate insecticides, but also using other pesticides. The use of combination of 2-5 types of pesticides and not evaluated in detail. As a suggestion, routine biomonitoring for pesticides is needed. This study found cellular and clinical disorder associate with the use of AChE inhibitor insecticides. Continuing education for farmworkers about the correct use of pesticides as well as self-protection and policy making by the government is needed to reduce poisoning due to improper use of insecticide.

Conclusion

Erythrocyte AChE activity associate with a history of pesticide exposure as well as symptoms or clinical findings in the field. In the cut-off value at 6.2 U/g hematocrit was related to hypertension. This cut-off value was easier to implement in practical setting because it only requires a measurement of AChE erythrocyte.

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