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A Correlation Study : Levels of Butyrylcholinesterase and Paraoxonase 1 Activity amongst Shallot Farmworkers in Brebes Regency, Central Java, Indonesia

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Abstrak

Insektisida organofosfat adalah jenis pestisida yang biasa digunakan pada kegiatan pertanian untuk membasmi atau melindungi tanaman dari serangan hama jenis serangga. Selain manfaat yang ditawarkan, insektisida organofosfat juga membawa ancaman bagi individu dan populasi. Petani bawang merah di Kabupaten Brebes adalah salah satu populasi berisiko untuk terpapar oleh insekstida organofosfat. Tingkat aktivitas Butyrycholinesterase (BuChE) dan Paraoxonase 1 (PON1) dalam darah memiliki peran penting sebagai penanda biologis untuk mengukur terjadinya paparan dan mengukur tingkat kerentanan tubuh akibat paparan insektisida organofosfat. Penelitian ini bertujuan untuk menganalisis korelasi antara tingkat aktivitas BuChE dan PON1 pada petani bawang merah di Kabupaten Brebes. Penelitian ini merupakan penelitian observasional menggunakan *cross sectional design*. Sebanyak 88 orang petani di Desa Dukuhlo, Kecamatan Bulakamba, Kabupaten Brebes, Indonesia dipilih secara acak sebagai sampel penelitian pada periode bulan April dan Mei 2017. Instrument penelitian menggunakan kuesioner untuk mengetahui karakteristik sosial demografik dari subjek penelitian. Pengambilan sampel darah dilakukan untuk menentukan tingkat aktivitas BuChE dan PON1. Sampel darah kemudian dianalisis di laboratorium Cito Tegal dan Gaky Undip Semarang. Analisis data menggunakan univariat dan bivariat (uji korelasi Rank Spearman). Tingkat aktivitas BuChE berkorelasi positif dengan tingkat aktivitas PON1 dengan kekuatan hubungan sedang (p=0,025; rho=0,238). Kesimpulan dari penelitian ini adalah terdapat korelasi positif antara kedua variabel tersebut. Petani dengan tingkat aktivitas PON 1 yang tinggi memungkinkan untuk mendetoksifikasi paparan akut insektisida organofosfat lebih baik. Penelitian lebih lanjut diperlukan untuk mengidentifikasi korelasi antara tingkat aktivitas PON1, kadar hormon tiroid, dan metabolit insektisida dalam urin.

Abstract

Organophosphate insecticides (OPs) are one of the pesticides commonly used in agricultural activities either to eradicate or to protect crops from insect attacks. Aside from the advantages proposed, this OPs substance also brings some worrisome threats for individual and population. Shallot farmworkers in Brebes Regency are population at risk to OPs exposure. The activity levels of Butyrylcholinesterase (BuChE) and paraoxonase 1 (PON1) in blood play important roles as a biomarker of exposure as well to measure the occurrence of OPs exposure in a human body and as a biomarker of susceptibility as well to measure the level of detoxifying OPs. The aim of this study was to analyse the correlation between levels of BuChE and PON1 activities amongst shallot farmworkers. A cross-sectional study was conducted on 88 male subjects selected randomly from Dukuhlo Village in Brebes Regency, Indonesia, occupationally exposed to OPs from April to May 2017. Using a structured questionnaire, a survey was carried out based on sociodemographic characteristics. Blood samples were collected to determine the levels of BuChE and PON1 activity. These samples were then analysed at laboratories of Cito in Tegal and Gaky, Undip in Semarang. Furthermore, data were analysed systematically using univariate and bivariate (a Spearman's Rank test). A significant correlation was found between these both variables (p=0.025 and rho=0.238) with slightly moderate positive relationship. To sum up, farmworkers with higher PON1 activity may have a better chance of detoxifying the acute effect of OPs exposure. A further research is needed to identify correlation between PON1 activity, levels of thyroid hormones, and OPs metabolites in urine.

INTRODUCTION

In Indonesia, organophosphate insecticides (OPs) were chemical compounds extensively used for agricultural purposes. The active substance of OPs commonly used by farmworkers in Indonesia was chlorpyrifos (Suhartono, *et al.,* 2012; Suratman, *et al.,* 2015). Farmworkers were a population at risk to OPs exposure. The occurrences of acute poisonings among farmworkers occupationally exposed to OPs have been investigated by many researchers around the world (Suratman, *et al.,* 2015). A study among 89 pesticide sprayers in Phnom Penh, Cambodia showed that most of the participants (88%) suffered from symptoms of acute OPs poisoning (Jensen, *et al.,* 2011). In addition, a study in 11 states of the USA during the period of 1998 – 2006 among 2,945 cases related to agricultural pesticide drift demonstrated that 47% were occupationally exposed at work, 92% suffered from low severity illness, and the overall incidence was 114.3 farmworkers (in million person-years) (Lee, *et al.,* 2011). A study in Turkey on 23 patients of acute OPs poisoning admitted to a hospital revealed that 65.2% of them were farmers (Soysal, *et al.,* 2011). Similarly, a study on 77 fruit farmworkers in Tainan City, Taiwan demonstrated that a majority of them (92.2%) had experienced one or more symptoms of acute poisoning due to OPs exposure (Weng & Black, 2015). BuChE inhibition is used as a biomarker of exposure to OPs and is used to monitor farmworkers at risk of OPs exposure (Balali-Mood & Saber, 2012). Accumulation of the neurotransmitter Acetylcholine (ACh) occurs due to OPs that irreversibly block acetylcholinesterase (AChE) in the synapse and cause over-stimulation of nicotinic and muscarinic receptors (Colovic, *et al.,* 2013; Lacasana, *et al.,* 2010). Some epidemiological studies conducted in agricultural workers demonstrated that exposure to OPs decreased levels of butyryl cholinesterase (BuChE) (Dhananjayan, Ravichandran, Anitha, & Rajmohan, 2012; Jamal, Haque, Singh, & Rastogi, 2015; Neupane, Jørs, & Brandt, 2017) and paraoxonase 1 (PON1) (Colovic, *et al.,* 2013; Lacasana *et al.,* 2010; Richard, *et al.,* 2013) activities. PON1 activity was a key enzyme to detoxify OP pesticides and was used as a biomarker of susceptibility (Munoz-Quezad, *et al.,* 2013).

Brebes Regency is an agricultural area located in Central Java Province, Indonesia that produces shallots as main commodities by which about 50% of farmworkers used chlorpyrifos (BPS-Statistics of Brebes Regency, 2016; Suratman, *et al.,* 2015). The aim of this study was to assess the correlation between the levels of cholinesterase and PON1 activities among shallot farmworkers.

METHOD

This was a cross-sectional study to evaluate the levels of BuChE and PON1 activity based on the use of OPs among shallot farmworkers. Brebes Regency of Central Java Province is one of the most intensive agricultural areas in Indonesia with shallots as main agricultural commodities in the area. Dukuhlo Village located at Bulakamba Sub-District with the most intensive agriculture was selected as a research site. Inclusion criteria for participant selection were: 1) that they were male; and 2) had to be employed in farm work within the past 3 months. These criteria were based on the following: 1) the majority of farmworkers in 2013 in Indonesia (24.36 million or 77%) (Indonesian Bureau of Statistics, 2013) were male; 2) engaging in farmwork within the past 3 months reflected recent likelihood of being exposed to OPs. In addition, complete recovery of BuChE as a biomarker of exposure to OP is 50 days (Mason, 2000). This study was conducted from April to May 2017, when pesticides were used less. As many as 88 eligible farmworkers had agreed to participate and signed informed consent forms. They were then interviewed and their blood samples were collected. Ethics approval was obtained from the Commission on Medical Research Ethics, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia with approval number: 1051/KEPK/III/2017.

ChE Gen 2 kit (PN 04498577190, Roche Diagnostics) on COBAS INTEGRA 800 was used for immediate BuChE determination. Enzymatic activity was obtained in kU/L with normal values ranging from 5.32 to 12.92 kU/L. Meanwhile, the ELISA method was performed on EL$_X$800 (BioTek® Instruments, Inc) to determine PON1 activity according to the manufacturer's instructions. All these biomarkers were analysed by laboratory analysts working in two accredited laboratories, namely Cito Laboratory located in Tegal City for BuChE analysis, and Gaky Laboratory of Diponegoro University located in Semarang City for analyses of PON1 activity.

In addition, a structured questionnaire was used to collect data of personal characteristics, the use of OPs, methods of OP application, types of personal protective equipment worn during OPs application, and clinical signs and symptoms related to OPs exposure. The levels of BuChE activity had a normal data distribution ($p=0.243$) whereas the data distribution of PON1 levels was not normal ($p=0.035$) using a Shapiro-Wilk test. Therefore, a Spearman's Rank test was performed to analyse a correlation between these both variables.

RESULTS AND DISCUSSION

Characteristics of the Study Population

The characteristics of the research participants are presented in Table 1. More than 50% of the farmworkers have graduated from elementary school. Notwithstanding, the percentage of the research subjects who were never attended school were quite high (24%). Meanwhile, the mean age of the farmworkers was 50.4 years old with the highest age was 70 years old. On an average, they had been working as a farmworker for 24.3 years and had length of work per day about 6 hours.

Table 1
Characteristics of the study population

Variables	Frequency (F)	Percent (%)
Level of education:		
Never attended school	21	24
Elementary School	50	57
Junior High School	11	12
Senior High School	5	6
Diploma (D1/D2/D3)	1	1
Age (year)	50.4 ± 9.8	(25-70)
Years as a farmworker	24.3 ± 11.6	(1-47)
Length of work per day (hour)	6.0 ± 1.5	(2-12)

Age, years as a farmworker, and length of work per day are expressed as mean ± standard deviation and minimum-maximum

The Types of OPs used in the Last Four Weeks

Table 2 presents the use of OPs by the research participants in the last four weeks to protect their crops. Almost half of the research subjects applied OPs to their crops in the last four weeks. The use of OPs was determined by identifying the active ingredients written on each label of pesticide container used by the participants.

Table 2
Use of OPs used in the last four weeks

Use of pesticides	Frequency (F)	Percent (%)
Yes	38	43
No	50	57

Last Time Applying Pesticides

Table 3 presents the last time applying pesticides by the participants. Almost half of the subjects applied OPs in the last 1-6 days ago.

Table 3
Last time applying pesticides

Last time applying pesticides	F	%
1-6 days ago	41	47
1-2 weeks ago	27	31
1 month ago	17	19
> 1 month ago	3	3

Methods of OPs Application

Methods of OP application among the participants are presented in Table 4. All research participants frequently used sprayer backpack to apply OP to their crops and poured OP into the sprayer application tank by hand (100%). In addition, nearly all subjects used equipment to stir the mixture when mixing OP such as dipper, tablespoon, and trowel (97%). On the other hand, almost half of the participants were against wind direction when spraying OP (49%).

Table 4
Methods of OP application

Activities	Frequency (F)	Percent (%)
Equipment used:		
Backpack sprayer	88	100
Ways to mix OPs:		
Pour into tank by hand	88	100
Equipment used to mix OPs:		
Hand/Arm	1	1
Stick/Paddle	1	2
Other	85	97
Ways to spray OPs:		
Wind direction	45	51
Against wind direction	43	49

Types of Personal Protective Equipment (PPE) frequently used

Types of PPE frequently used by farmworkers during working with OP are shown in Table 5.

Table 5
Types of PPE usually worn during working with OP

Types of PPE worn	Frequency (F)	Percent (%)
Clothes:		
Long sleeved shirt	79	90
Short sleeved shirt	9	10
Pants:		
Long pants	47	53
Short	41	47

Types of PPE worn	Frequency (F)	Percent (%)
Headwear:		
Wide brim hat	9	10
Cap	78	89
No hat	1	1
Footwear:		
Waterproof boots	1	1
No shoes	87	99
Mask:		
Cloth mask	34	39
No mask	54	61
Gloves:		
Waterproof elbow length gloves	2	2
Cloth gloves	1	1
No gloves	85	97
Eye protections:		
No eye protection	88	100

Most of the research participants were reported usually wearing long sleeved shirt (90%), wearing long pants (53%), wearing cap (89%), not wearing footwear (99%), not wearing mask (61%), not wearing gloves (97%), and not wearing eye protection (100%) when working with OP.

Levels of BuChE and PON1 activities

Mean levels of BuChE and PON1 activities respectively were 7.7 kU/L and 8.9 ng/ml (Table 6).

Table 6
The levels of BuChE and PON1 activities

Biomarkers	mean±sd	Minimum	Maximum
BuChE (kU/L)	7.7±2.2	1.6	13.4
PON1 (ng/ml)	8.9±2.5	3.7	14.0

BuChE activity was positively correlated with serum PON1 activity ($r = 0.238$, $p = 0.025$) among farmworkers (Fig 1).

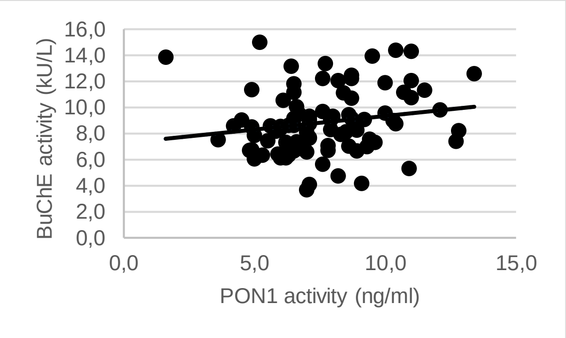

Fig 1
Correlation between BuChE and PON1 activities using a Spearman's Rank test (rho = 0.238, p = 0.025)

OP-related Symptoms

A percentage of the participants complaining OP-related symptoms in the last four weeks is presented in Table 7. Some research participants reported OP-related symptoms like weakness (3%), headache (8%), dizziness (19%), emesis (6%), blurred vision (15%), lacrimation (5%), and muscle weakness (2%).

Table 7
OP-related symptoms

Symptoms	Frequency (F)	Percent (%)
Weakness:		
Yes	3	3
No	85	97
Headache:		
Yes	7	8
No	81	82
Dizziness:		
Yes	17	19
No	71	81
Emesis:		
Yes	5	6
No	83	94
Blurred vision:		
Yes	13	15
No	75	85
Lacrimation:		
Yes	4	5
No	84	95
Muscle weakness:		
Yes	2	2
No	86	98

DISCUSSION

Our study found 38 of 88 research participants (43%) applied OPs compounds particularly chlorpyrifos to their crops (Table 2) and most participants (47%) said that they used OPs for the last time within one to six days before being collected blood samples (Table 3). The length to recover from mild inhibition has been shown to be about 1-3 days whereas recovery from moderate inhibition is 1-2 weeks (Workplace Health and Safety Queensland, 2012).

A study conducted in South India among 28 agricultural workers occupationally exposed to OPs and 13 unexposed male workers as the reference group demonstrated that the levels of BuChE significantly declined about 56% among the exposed workers (Dhananjayan *et al.*, 2012).

A study among male farmers in South Korea demonstrated that lifetime days of OPs application could increase risk of acute OPs poisoning (OR=1.74; 95%CI=1.32-2.29) (Kim *et al.*, 2013). PON1 has a capacity to hydrolyse the active metabolites of OPs including parathion, diazinon and chlorpyrifos specifically in the central nervous system (Costa, Giordano, Cole, Marsillach, & Furlong, 2013). In addition, PON1 also play an important role in protecting a human body from some diseases related to oxidative stress like diabetes mellitus (Kulka, 2016).

In our study, the levels of BuChE statistically significantly correlated with the levels of PON1 activity. The finding of this study is consistent with the results of (Richard *et al.,* 2013). They found that serum PON 1 positively correlated with cholinesterase activity among persons who consumed OPs and committed to suicide. In contrast, other studies showed that BuChE did not correlate with PON1 activity (Albers, Garabrant, Berent, & Richardson, 2010; Ellison *et al.,* 2012; Gonzalez *et al.,* 2012).

The use of OPs in accordance with the pesticide labels is very important to be obeyed by farmworkers or pesticide applicators. Adverse health effects due to improper use of OPs often occur from acute to chronic conditions in around the world (Suratman, Edwards, *et al.,* 2015). The inhibitions of both BuChE and red blood cholinesterase, known as AChE, by OPs compounds provide information of the OPs exposure in an individual (Marsillach *et al.,* 2011). Furthermore, the levels of PON1 also need to be evaluated to determine individual susceptibility to OPs like chlorpyrifos (Albers *et al.,* 2010).

BuChE and PON1 are two kinds of enzymes that can be found in the liver and plasma (Albers *et al.,* 2010; Tvarijonaviciute *et al.,* 2012). BuChE, also known as plasma cholinesterase (PChE) is included in the same structural class of protein like Acetylcholinesterase (Richard *et al.,* 2013). OPs inhibit the activity of cholinesterase in hydrolysing ACh, a neurotransmitter, into choline and acetate acid in order to prevent over-stimulating post-synaptic nerves, muscles, and exocrine glands (King & Aaron, 2015). BuChE is a sensitive biomarker to OPs exposure particularly chlorpyrifos (Hofmann *et al.,* 2010). A biological half-life of chlorpyrifos is very short about 62 hours in fat tissue and 18 hours in plasma (Suratman, Edwards, *et al.,* 2015).

In addition, clinical effects of OPs poisoning due to acetylcholine excess were suffered by some research participants, namely weakness, headache, dizziness, emesis, blurred vision, lacrimation, and muscle weakness (Table 7). It means that over-stimulation of muscarinic and nicotinic receptors had occurred. Clinical signs and symptoms commonly occurred on muscarinic receptors due to the inhibition of cholinesterase by OPs are sweating, lacrimation, constricted pupils, wheezing cramps, excessive salivation, vomiting, diarrhoea, tenesmus, blurred vision, bradycardia, and urinary incontinence (King & Aaron, 2015). These signs and symptoms are also known as respiratory manifestations. On the other hand, clinical signs and symptoms due to ACh excess on nicotinic receptor encompass fasciculations, weakness, cramps, paralysis, twitching, respiratory embarrassment, cyanosis, tachycardia, and arrest (King & Aaron, 2015). These signs and symptoms are recognised as muscular manifestations.

All farmworkers in the research site used backpack tank sprayer to apply OPs on their crops. Unfortunately, they poured OPs into tank using their hands (Table 4). In addition, most of them also did not wear proper PPE during working with OPs compounds (Table 5). This conditions potentially increased amount of OPs that entered their bodies through dermal absorption. Even though 57% of the research participants did not use OPs in the last four weeks, the OPs exposure among them is still possible to occur. Unwitting exposure to OPs can happen when they visited their farming areas, touched crops that recently sprayed using OPs, inhaled air at the farming area, wore unwashed clothes after farming activities (Suratman, Edwards, *et al.,* 2015). OPs could enter into the body through ingestion, inhalation, as well as absorption on the skin (Khan, Hashmi, Mahjabeen, & Naqvi, 2010).

CONCLUSION

BuChE and PON1 activities in the serum of the shallot farmworkers were measured and positively and significantly correlated. Farmworkers with higher PON1 activity may have a better chance of detoxifying the acute effect of OPs exposure. Farmworkers in Brebes Regency need to change their methods in applying OPs and to wear PPE properly in order to prevent the increase of OPs exposure. Further research is needed to investigate the correlation between PON1 activity, the levels of thyroid stimulating hormones, and the levels of OPs metabolites in urine samples.

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