RESEARCH ARTICLE

CHOLINESTERASE ACTIVITY IN PALM OIL PLANTATIONWORKERS IN PARENGGEAN, KOTAWARINGIN TIMUR

Suratno, Dwi Purbayanti & Hildayanti

Department of Medical Laboratory Technology, Faculty of Health Sciences,
Universitas Muhammadiyah Palangkaraya

e-mail: nono.suratno89@yahoo.com

ABSTRACT

Use of pesticide is widely used in palm oil plantation industry. Pesticide exposure can affect pesticide-spraying workers's health.This study aimed to determine asetylcholinesterase enzyme (AChE) activity levels and identify of poisoning risk factors on pesticide-spraying workers in a palm oil plantation Comanditaire Venootschap (CV) unit in Parenggeran, Kotawaringin Timur, Central Kalimantan. Cross-sectional study design was conducted to examine cholinesterase enzyme activity levels on 15 pesticide-spraying workers. The cholinesterase levels was measured using an automated photometric technique. This study found that all of workers have normal cholinesterase activity levels. The average cholinesterase activity levels was 7065,36 U/L and 6528 U/L, respectively for male and female workers.It was also found that two male workers cholinesterase activity levels of using glyphosate pesticide was almost close to the lower limit of the normal levels range, 4720 U/L and 4958 U/L, respectively. Use of Personal Protective Equipment (PPE) during the pesticide spraying and maximum time allowed of pesticide spraying in a day have to be considered as important rule to avoid excessive pesticide exposure.

Keywords: Cholinesterase, pesticide exposure, palm oil plantation workers

INTRODUCTION

Synthetic pesticides are part of agrochemicals widely used in most agricultural areas to increase crop yields, including palm oil plantation.The most used are organophosphates (OP), glyphosate and carbamates. The widespread use of those pesticides throughout the world exposes to poisoning by these chemical agents, particularly in developing countries (Yapo et al., 2017). Indonesia is a developing country rising palm oil plantation sector, including in Parenggean, Kotawaringin Timur, Central Kalimantan.The use of pesticides in this industry continues to increase.

Those pesticides are considered safe when used under a limitation level with proper application procedure. The improper use of pesticides can affect on worker's health. Anwar (1997) explained that pesticide uptake occurs mainly through the skin and eyes, by inhalation, or by ingestion.

An indicator of pesticide poisoning in worker's body is cholinesterase activity in blood.Acetylcholinesterase enzyme (AChE) catalyzes degradation of the neurotransmitter acetylcholine in synaps. Organophosphate pesticides phosphorylate acetyl-

choline, thereby, reduce the ability of the enzyme to break down the neurotransmitter. This causes accumulation of acetylcholine in the central and peripheral nervous systems, resulting in an acute cholinergic syndrome via continuous neurotransmission (Nadiah et al., 2015). AChE may serve as a biomarker for assessing individual or population-group exposure to organophos-phate and carbamate pesticides (von Osten et al., 2004).

Blood AChE activity testing is considered a relatively simple procedure, it is used for assessing the extent of human exposure to pesticide.If performed and interpreted correctly, AChE testing can be an important instrument for preventing or diagnosing pesticide poisoning (Lessenger & Reese, 1999).

This study aimed to asses cholinesterase activity levels and identify of poisoning risk factors on pesticide-spraying workers in a palm oil plantation in Parenggean, Kotawaringin Timur, Central Kalimantan.

METHODS

Cross-sectional study design was conducted to examine cholinesterase enzyme activity on palm oil plantation workers. This study was carried out in a palm oil plantation Comanditaire Venoots-chap (CV) unit in Parenggean, Kotawaringin Timur district, Central Kalimantan during April to June, 2017. Cholinesterase activity levels were determined in Clinical Laboratory, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya.

Purposive sampling technique was used in this study, sample was collectedbased on some certain purposes and considerations.The inclusion criteria of the sample must be: (a) Male or female who had been working longer than one year as a pesticide spraying worker, (b) in good health and did not have associated disease with liver damage, and (c) disposed to be a respondent. The palm oil plantation workers who meetingthose criteria could complete the questionnaire and undergo the AChE test.

Tools and Materials

Materials were used in this study consist ofserum sample, CholinesteraseTest kit: Reagent A(pyrophosphate 95 mmol/L, hexacyanoferrate(III) 2,5 mmol/L, pH 7,6) and Reagent B (butyrylthiocholine 60 mmol/L), andalcohol swab 70%.

This study used some tools such as syringe 3 cc, micropipette(1000 µL, 50 µL, 20 µL, dan 5 µL), blue micropipette tips, white micropipette tips, test tube12x75 mm and centrifuge. Cholinesterase assay was determined using Photometers (5010 V5+ Robert Riele).

Data and Sample Collection

The data obtained during the questionnaire and the AChE assay of the pesticide users.

The questionnaire items included general information, Personal Protective Equipment (PPE) usage, working period in pesticide spraying, exposure period during spraying in a day, and type of pesticides used.

The cholinesterase enzymelevel was obtained using an automated photometric technique.The principle of the assay is that Cholinesterase(CHE) catalyzes the hydrolysis of butyrylthio-choline to thiocholine and butyric acid.The catalytic concentration is determined from the rate of decrease of hexacyano-ferrate(III), measured at 405 nm.

The blood sample collection involved venipuncture procedure based on Depart-ment of Health of Republic of Indonesia 2008. The blood collected by venipuncture was collected in dry vacutainer tubes for the determination of AChE.

Serum processing referred to Good Laboratory Practiceguidelines Department of Health of Republic of Indonesia 2004. Blood sample was prior allowed at room temperature for 20-30 min, and were centrifuged at 3000 rpm for 5-15 min.The determination of cholinesterase level in serum was performed within 2 h after sample collection. No hemolysis and lipemic serum were analyzed.

RESULTS AND DISCUSSION

Socio-demographic characteristics of the sample

The study population consisted of 19 workers, and who met the criteria was 15 workers. The sample of this study comprised of 11 men and 4 women. The sex ratio (male/female) was equal to 2.75.The sample was relatively young: 60% of the questioned persons were aged 30 to 40 years, and the rest 40% were aged 40 to 50 years. In addition, 40% of the population or six workers had 5 years of pesticide-spraying experience(**Table 1**).Yapo et al. (2017) found all workers (100%) were male.It means that workers handling the plant protection products dominated by men.

Tabel 1 Characteristics of the samples

Characteristics	Variables	Number	%
Sex	Male	11	73.3
	Female	4	26.7
Age category, y	30-40	9	60
	40-50	6	40
Working period in pesticide spraying, y	1	3	20
	2	4	26.7
	3	2	13.3
	5	6	40

Table 2 Identification of pesticide poisoning risk factors

Risks Factors	Variables	Number	%
Use of PPE during the pesticide spraying	Always	9	60
	Occasionally	6	40
Exposure period during pesticide spraying, h	4	7	46.7
	5	8	50.3
Last pesticide spraying, d	<7	8	50.3
	>7	7	46.7
Active ingredient in Pesticide used	Glyphosate	7	46.7
	Paraquat	8	50.3

Identification of poisoning risk factors

The main pesticide poisoning risks factors were identified that 60% of the workers always wear the personal protective equipment (PPE) during pesticide spraying, such as gloves, hat, mask and boots. Workers exposed to pesticice for 5 h during pesticide spraying were 50.3%. In addition, 46.7% of the workers applied pesticices with active ingredient glyphosate, and 50.3% of the workers used paraquatpesticide (**Table 2**).

C**holinesterase activity levels**

The normal range for blood cholinesterase levels were classified according to thegender ranging from 4620-11.500 U/L for men and 3930-10800 U/L for women. The cholineste-rase activity levels was considered abnormal or decrease when lower than the normal range.This study found that all of workers were normal. The average cholinesterase activity levels was 7065.4 U/L and 6528 U/L, respectively for male and female workers (**Table 3**).

Table 3 Cholinesterase acticity levels of the samples

No.	Initial Name	Sex	Active ingredient in pesticide used	Cholinestera-se levels(U/L)	AverageCholines-terase levels(U/L)
1	A	Male	Glyphosate	8816	
2	R	Male	Glyphosate	7139	
3	M	Male	Glyphosate	4720	
4	K	Male	Glyphosate	4958	
5	M	Male	Paraquat	8159	
6	F	Male	Paraquat	6811	**7065.4**
7	R	Male	Paraquat	5666	
8	A	Male	Paraquat	8622	
9	D	Male	Paraquat	9772	
10	U	Male	Paraquat	6909	
11	M	Male	Paraquat	6147	
12	S	Female	Glyphosate	4870	
13	L	Female	Glyphosate	7169	**6528**
14	B	Female	Glyphosate	8022	

15	D	Female	Paraquat	6051

Table 3 also indicates that cholinesterase activity levels of two male workers using glyphosate pesticide was almost close to the lower limit of the normal levels range. The low cholinesterase activity levels were 4720 U/L and 4958 U/L, respectively.Low blood cholinesterase concentrations indicatehigh exposure to pesticide (Nadiah et al., 2015). But, a diagnosis of poisoning can be made by comparing post-exposure AChE levels with baseline (pre-exposure) AChE levels. If there is no baseline AChE level recorded, and if the offending chemical is in question, the clinician must base treatment on the clinical signs and symptoms (Lessenger & Reese, 1999).

Table 4 shows that the average cholinesterase acticity levels on male workers using pesticide with glyphosate was 6408.3 U/L, lower than who used pesticide with paraquat 7440.9 U/L. On the contrary, the average cholinesterase acticity levels on female workers using pesticide with glyphosate was 6687 U/L, higher than who used pesticide with paraquat 6051 U/L. According to the average cholinesterase levels, workers used glyphosate pesticides has lower level than workers used paraquat pesticide. In another words, that pesticide with glyphosate as an active chemicals has stronger inhibition of cholinesterase. El-Demerdash et al. (2001) reported that the inhibitory effect of glyphosate on the activities of AChE was stronger than paraquat. This in vitro study found that at same the concentrations, glyphosate lead the 44.1% inhibition values of AChE activity and the ID_{50} to AChE was 714.3 mM, higher than paraquat which only lead mild inhibitory effect on the AChE activity around 20% and the ID_{50} to AChE was only 77.7 mM. Another study also revealed that certain enzymes, such as cholinesterase activity in serum and red blood cells influenced by pesticide exposure (Awad et al., 2014).

The glyphosate (N-phosphonomethyl-glycine) is a post-emergent organophosporus herbicide widely used in several types of cultures.The glyphosate is a broad-spectrum, nonselective, and systemic herbicide. (Demerdash et al., 2001; Modesto & Martinez, 2010).

Table 4 Average cholinesterase acticity levels based on active ingredient in pesticide used

Active ingredient in pesticide used	Sex	Number	Normal Cholinesterase levels (U/L)	AverageCholinesterase levels (U/L)
Glyphosate	Male	4	4620-11500	6408.3
	Female	3	3930-10800	6687
	All	7		6527.7
Paraquat	Male	7	4620-11500	7440.9
	Female	1	3930-10800	6051

	All	8	7267.1

Table 5 Average cholinesterase activity levels based on working period

Working period, year(s)	Sex	Num-ber	AverageCholineste-rase levels (U/L)
1	Male	2	7813.5
	Female	1	4870
	All	3	6832.3
2	Male	2	6048.5
	Female	2	7595.5
	All	4	6822
3	Male	1	5666
	Female	1	6051
	All	2	5858.5
5	Male	6	7388.2
	Female	-	-
	All	6	7388.2

This study found that cholinesterase levels on pesticide-spraying workers had no relation to working period. This is shown by**Table 5**. Lestari & Anggraini (2016) reported that there is no relation between working period and cholinesterase blood levels among 102 pesticides-spraying workers in a palm oil plantation company in the same district, Kotawaringin Timur.

Table 6 Average cholinesterase activity levels based on use of PPE

Use of PPE during spraying	Sex	Num-ber	AverageCholineste-rase levels (U/L)
Always	Male	6	7538.5
	Female	3	6687
	All	9	7254.7
Occasiona l-ly	Male	5	6497.6
	Female	1	6051
	All	6	6423.2

Table 6 indicates that the average cholinesterase activity levels in workers who always used PPE during pesticide spraying was higher levels than workers who used PPE occasionally.Hinson et al. (2017) also reported that regarding the wearing of PPE, those who did not wear it showed more inhibition of AChE. The lack of PPE usage considerably influenced acetylcholinesterase percentage.

CONCLUSIONS

This study revealed that palm oil plantation in Parenggean, Kotawaringin Timur used pesticide with active ingredient glyphosate and paraquat. The most dominant workers were men (73.3%), relatively young (100% of the workers were aged under 50 years), with working period varies from one to five years experience in pesticide spraying. All of workers have normal cholinesterase activity levels. The average cholinesterase activity levels was 7065,36 U/L and 6528 U/L, respectively for male and female workers. It was also found that two male workers cholinesterase activity levels of using glyphosate pesticide was almost close to the lower limit of the normal levels range, 4720 U/L and 4958 U/L, respectively. Use of Personal Protective Equipment (PPE) during the pesticide spraying and maximum time allowed of pesticide spraying in a day have to

be considered as important rule to avoid excessive pesticide exposure.

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