



The Correlation Between Pesticide Exposure and Malondialdehyde Levels on Onion Farmers in Karanganyar

Anamika Labitta¹, Budiyo Budiyo², Mursid Rahardjo³

^{1,2,3}Master of Environmental Health, Faculty of Public Health, Diponegoro University

Prof. H. Soedarto SH Street, Tembalang, Semarang, Central Java, Indonesia

Email : amlabitta@students.undip.ac.id

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ABSTRACT

Pesticides could induce oxidative stress, which started as formation of free radicals and alteration of antioxidant, enzymatic system, as well as lipid peroxidation. The occurrence of lipid peroxidation could be assessed by measuring malondialdehyde (MDA) serum level. This study's objective was to identify the correlation of previous pesticide exposure with MDA levels in onion farmers in Karanganyar. This study was an observational analytic study which used cross sectional design. Study population was a total of 200 farmers. Total sample of this study is 53 male farmers respondents. Data was analyzed using univariate and bivariate analysis with Rank Spearman and Mann-Whitney test. The average level of cholinesterase

was 6409,36 U/L, whereas the average of MDA levels according to the cut-off point was 206,41 ng/ml. The study results showed that there was a low correlation between cholinesterase levels and MDA levels, in which the correlation was in inverse manner, meaning that the higher the cholinesterase levels, the lower the MDA levels ($p= 0,029$, $r= -0,300$). There was also a difference in regards to the average cholinesterase levels between farmers who was not fully equipped with personal protective equipment (PPE) and farmers who was not fully equipped ($p= 0,027$). The non-fully equipped with PPE group showed rank average of 22,90, which was lower compare to fully equipped group which showed rank average of 32,35. This study concluded that the cholinesterase levels were significantly correlated with MDA levels and there was an average difference between the 2 PPE-equipped farmer groups

Keywords: *Cholinesterase, Free Radical, Malondialdehyde, Oxidative stress, Pesticide.*

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Introduction

Pesticides which enter the body and formed a reaction could resulted in formation of Reactive Oxygen Species (ROS). ROS could induce oxidative stress by causing lipid peroxidation resulting in various illness and health disorders as cells and nervous system damages. (Winarsi, 2005) Malondialdehyde (MDA) is the main product to evaluate the occurrence of lipid peroxidation. A study conducted on farmers in Egyptian agricultural region to identify the correlation between organophosphate exposure with oxidative stress using MDA biomarker showed that MDA levels were significantly higher in farmers who were exposed with organophosphate compared to the non-exposed group. MDA levels were also significantly correlated with duration of work (years), number of work hour per day. An incomplete usage of PPE increased the risk of higher MDA level (Elshamy et al., 2019) Karanganyar is an agricultural area which produce onions. The preliminary study that was done previously showed that farmers in Karanganyar usually sprayed pesticides three times a week using a mixture of 2 to 3 types of pesticides. The aim of this study is to identify the correlation between previous exposure of pesticides and MDA levels on onion farmers in Karanganyar.

Methods

This study was an observational analytic study with a cross sectional design. The population study is a total of 200 male and female farmers which reside in Kalisoro Karanganyar. Sample collection was being done using random sampling technique with inclusion criteria such as male onion farmers that used chemical pesticides, with age less than 65 years old, and were not suffering from any degenerative disorders. The samples in this study was 53 male farmer respondents. This study was conducted in Kalisoro Village Karanganyar on July 2019. Primary data was obtained through measurement of cholinesterase levels in regional health laboratory in Semarang and measurement of MDA levels in GAKI Laboratory Faculty of Medicine Diponegoro University. Interviews were also being done to identify respondents characteristic and previous exposure to pesticides. Secondary data such as agricultural data from Karanganyar Regency Agricultural Department and monography data from Kalisoro Village Tawangmangu District Karanganyar Regency Government Office was also obtained.

Results

1) Characteristics of Respondents

Table 1. Characteristics of Respondents

Variables (n = 53)	Mean (SD)	Median (Min-Max)	f	%
Age	52,98 (10,48)	55,00 (27-64)		
Body Weight (kg)	55,92 (8,04)	55,00 (39-80)		
Body Height (cm)	157,38 (6,18)	158,00 (142-175)		
Work Time (year)	27	30 (2-55)		
Work Time (year)				
≥27 years			28	52,8
<27 years			25	47,2



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Variables (n = 53)	Mean (SD)	Median (Min-Max)	f	%
Work Duration	46,75	49,0 (14-70)		
Work Duration				
≥49 hours per week			28	52,8
<49 hours per week			25	47,2
Spraying Frequency				
Often (≥ 2 times a week)			25	47,2
Rarely (< 2 times a week)			28	52,8
Usage of Personal Protection Equipment				
Incomplete			30	56,6
Complete			23	43,4
Mixing method				
Directly by hands			0	0
Using a mixer tool			53	100
Amount of mixture				
> 2 types of pesticides			29	54,7
Single pesticide			24	45,3
Smoking				
Yes			16	30,2
No			37	69,8
Drugs/Herbal Consumption				
Yes			33	62,3
No			20	37,7

The age average of respondents was 52,98 years, with the youngest being 27 years and the oldest respondents was 64 years old. Respondents average work time was 27 years, in which the shortest was 2 years and the longest time was for 55 years. Each week the average respondents spent approximately 46,75 hours per week in the fields. A total of 25 respondents (47,2%) sprayed pesticides no less than 2 times weekly and 28 respondents (52,8%) sprayed for less than 2 times a week. As much as 56,6% respondents were not fully equipped with PPE at work. All respondents used mixing tool when mixing the pesticides.

As much as 29 respondents (54,7%) used more than 2 types of pesticides, whereas 24 respondents (45,3%) used a single pesticide. Respondents who were a smoker was 16 respondents (30,2%) which was lower than the non-smoker ones which was 37 respondents (69,8%). A total of 33 respondents (62,3%) had the habits of consuming either medications or herbals daily.

2) Results of Cholinesterase and MDA Serum Levels Laboratory Measurement

Table 2. Results of Cholinesterase and MDA Serum Levels Laboratory Measurement

Variables	Mean	SD	Median	Min	Max	Normal Level
Cholinesterase (U/L)	6409,36	1479,77	6131,00	4452	10862	4620-11500
Malondialdehyde (ng/ml)	206,41	75,42	209,60	34,7	371,0	



The average of cholinesterase serum level was 6409,36 U/L with the minimum value of 4452 U/L and maximum value of 10862 U/L. Central Java Province Health Laboratory and Testing Center for Medical Devices established the normal level for cholinesterase measurements as 4620-11500 U/L. In regards to the measurements of malondialdehyde levels, this study results showed that the average of MDA levels was 206,41 ng/ml, with the minimum value of 34,7 ng/ ml and maximum value of 371 ng/ml.

3) Correlation Between Cholinesterase and MDA Levels

Table 3. Correlation between Cholinesterase and Malondialdehyde (MDA) Levels

Variable	<i>Malondialdehyde (MDA)</i>	
	R	p
<i>Cholinesterase</i>	-0,300	0,029

There was a significant correlation between malondialdehyde (MDA) and cholinesterase levels. The correlation coefficient was -0,300, indicating a low correlation between cholinesterase levels and malondialdehyde levels, and was a negative correlation, meaning that the variables move in opposite directions – the higher the cholinesterase levels, the lower the malondialdehyde levels would be, and vice versa.

4) Correlation Between Work Duration and Work Time with Cholinesterase Levels

Table 4 Correlation Between Work Duration and Work Time with Cholinesterase Levels

Variable	<i>Cholinesterase</i>	
	R	p
Work Duration	-0,060	0,672
Work Time	-0,215	0,122

According to table 4, there was no significant correlation between work duration and cholinesterase levels. There was, however, a low correlation between work duration and cholinesterase levels, a negative correlation in opposite direction, showing that the longer the duration of work, the lower the cholinesterase levels would be, and vice versa. In regards to work time and cholinesterase levels, there was no significant correlation between the two variables. Correlation coefficient was -0,215, indicating that this value was in opposite directions, hence the longer the work time, the lower the cholinesterase levels, and otherwise.

5) Average Difference of Spraying Frequency, PPE Usage, and Amount of Pesticide Mixture with Cholinesterase Levels



Table 5. Average Difference of Spraying Frequency, PPE Usage, and Amount of Pesticide Mixture with Cholinesterase Levels

Variables	Mean Rank	p
Spraying Frequency		
“Often” ≥ 2 times a week (n= 25)	31,22	0,060
“Rarely” < 2 times a week (n= 28)	23,23	
PPE Usage		
“Incomplete” (n= 30)	22,90	0,027
“Complete” (n= 23)	32,35	
Amount of Mixture		
“> 2 types of pesticides” (n= 29)	29,78	0,150
“Single pesticide” (n= 24)	23,65	

According to the statistic test results showed in table 5, we obtained the p value = 0,060 which showed that there was no significant difference on average of cholinesterase levels between farmers who sprayed pesticide twice or more times a week and farmers who sprayed less than that. In APD usage variable, we discovered p value = 0,027 which indicated a significant difference on average cholinesterase levels between farmers who were not fully equipped with personal protection equipment (PPE) and farmers who were fully equipped. There was no difference on average cholinesterase levels in both farmers who used more than 2 types of pesticides and those who used a single pesticide.

Discussion

Pesticide is a chemical substance which could inhibit the activity of cholinesterase. Inhibition of AChE leads to accumulation of acetylcholin and prolonged action of acetylcholines in nervous system; both neuromuscular junction and glandular nerves. This accumulation resulted in cholinergic respons such as muscle contraction. Organophosphate induced oxidative stress which in turn, increased the Reactive Oxygen Species (ROS) production and weakened antioxidants.(Lukaszewicz, 2010)

Decreased cholinesterase levels have been associated with increasing occurrence of lipid peroxidation within the brain. This indicated a damage to the molecules and degeneration of the nerve cells (neurons).(Arif, 2014) Statistical analysis showed, that if the cholinesterase levels continue to decrease in farmers exposed with pesticides, the higher their MDA levels would be. This also supported the study by Surajudeen, on 60 farmers in Nigeria, which showed a significant correlation between AChE levels and oxidative stress which was indicated by the MDA serum levels ($p = 0,033 < 0,05$, $r = -0,39$).(Surajudeen et al., 2014)

This finding is consistent with a study by Rustia in 2010, which also showed that there was no significant correlation between work duration per day with cholinesterase levels



($p = 0,76$). (Rustia et al., 2010) Work duration caused a difference in exposure intensity and the amount of absorbed pesticides on each farmers. A continuous and prolonged exposure was found to be more harmful than intermitten exposure at one time. Spraying farmers whom repeatedly exposed to pesticides in a long period could develop a chronic intoxication. Pesticides with a high toxicity in a low amount resulted in fewer damage compared to pesticides with a low toxicity but in high amount. (Raini, 2007) Pesticides exposure in a short period could cause a mild intoxication in farmers, such as cholinesterase inhibition after organophosphate exposure could developed in 2 weeks and could last up to 6 weeks. Carbamate pasticides have a reversible impact, the inhibitory effect on cholinesterase is transient and will resolved in few hours, hence carbamate pesticides could not cause a chronic intoxication. In this study, pesticides intoxication on farmers did not seem to be affected by work duration daily, as farmers who stopped using pesticides for a long period will resulted in the detachment of pesticide molecules in blood. (Runia, 2008)

The longer the work time of a farmer, the lower also the cholinesterase serum level in blood would be, as more work time means increased time of contact with pesticides and risk of intoxication. The correlation between cholinesterase levels and work time may be dependent on how the farmers protect themselves from exposure and the type of pesticides used. This result is consistent with the study by Budiawan which was conducted on onion farmers in Pati, which showed that there was no correlation between work time and cholinesterase levels ($p = 1,000$). (Budiawan, 2013)

The main entry point of pesticides into the body is through the skin and respiratory system. Pesticides contamination on the skin is a very common thing to happened, and when sweating the absorption of pesticides which came in contact with the skin became more effective. Therefore, using an appropriate protective clothes, mask, and hand gloves is necessary to prevent the entry of pesticides exposure into the body. This finding is consistent with the study by Marsaulina, which showed that there was a correlation between PPE and the incidence of pesticide intoxication by measuring cholinesterase serum levels. Statistical analysis results showed that farmers who were not fully-equipped with PPE were 5,3 times more likely to develop pesticides intoxication than farmers who fully-equipped with PPE. (Marsaulina, 2007)

A higher spraying frequency will caused farmers to be exposed by pesticides more often, making them more susceptible for intoxication. The recommended spraying frequency is maximum twice in one week. Pesticides exposure in short interval but with high frequency caused the pesticide residues in the body to be higher. (Annida, 2018) The different finding in this study could be explained by variation in factors of pesticides exposure such as types of pesticides used in the mixture and PPE usage. No correlation between spraying frequency and cholinesterase levels which showed in this study may be because this study was coincidentally conducted when some farmers were just entering harvesting time and beginning to start planting new seeds, hence pesticides spraying was done quite rarely. Furthermore, this study was also conducted in dry season, where the pesticides spraying was not done as often as in raining season, which in turn affected intoxication rate in farmers. This wasn't consistent with study by Zulmi, which showed a correlation between spraying frequency and cholinesterase levels ($p = 0,042$). (Zulmi, 2016)



Several studies have reported that the impact in health problems caused by pesticides mixture was equivalent with only the most active component. This study showed no correlation between amount of pesticides mixture and cholinesterase levels which may be because the farmers already knew how to properly mixed the pesticides, which was using protective equipment such as mask and hand gloves, and stirring the mix with mixing tool, and not by hand, hence minimalizing pesticides exposure into the body. The presence of antioxidant might also affected the occurrence of stress oxidative within the body. This is consistent with a study by Yuantari, which showed there was no correlation between amount of pesticides in the mixture with the incidence of pesticides intoxication ($p = 1,00$). (Yuantari, 2009)

Conclusion

There was no significant correlation between cholinesterase levels with MDA levels ($p = 0,029$, $r = 0,300$). There was no significant correlation between cholinesterase levels with farmers' duration of work ($p = 0,672$, $r = -0,060$). There was also no significant correlation between cholinesterase levels with farmers' work time ($p = 0,122$, $r = -0,215$). There was no difference in average cholinesterase levels between farmers group with spraying frequency more than 2 times a week and farmers group who rarely sprayed pesticides or less than 2 times a week ($p = 0,060$). There was an average difference in cholinesterase levels between farmers with complete personal protective equipment (PPE) and farmers with incomplete ones ($p = 0,027$). There was no difference in average cholinesterase levels between farmers using more than 2 types of pesticides and farmers who used only one type of pesticide in one spraying activity ($p = 0,150$).



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