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# The Correlation between Pesticide Exposure and Glutation Peroxidase (GPx) Levels on Onion Farmers in Karanganyar

Pradipta Dhimasrastha S.P[1], Budiyono Budiyono[2], Mursid Raharjo[3]

Master of Environmental Health, Faculty of Public Health, Diponegoro University, Semarang, Indonesia
Corresponding author: Pradipta.dhio@yahoo.com

***Abstract*— *Pesticides have been widely used in agriculture and its usage is quite prevalent. Pesticides are one of many chemical substances that may induce the formation of free radicals. This study aims to investigate the correlation between previous pesticide exposure and Glutathion Peroxidase (GPx) levels on onion farmers in Karanganyar District. It is an observational analytic study with a cross-sectional design. A sample size of this study is 53 male farmers respondents. Variables were obtained by interviews, questionnaire and blood samples. Approximately 4 ml of venous blood were taken to obtain ± 1,5 ml blood serum each for two variables, including cholinesterase variables measure with Kinetic photometric test in Labkesda Semarang and GPx variable measured with Glutathione peroxidase Elisa Kit in GAKI Lab Semarang. Data were analyzed using Rank Spearman and Mann-Whitney test with α=0,05. Mean cholinesterase level was 6404,85 U/L with reference 4620-11500 U/L whereas mean GPx level was 14,1468 ng/ml with reference 0,5-10 ng/ml. Descriptive test showed 56,6% of farmers were not fully equipped with PPE, 47,1% farmers did not take a shower after spraying and 28,3% did not change their clothes after spraying. Study results showed that there was a negative correlation between cholinesterase level and GPx level (p= 0,024, r= -0,310). There was also a mean difference between PPE usage (p= 0, 027), Showering activity (p =0,042), Clothes change (p =0,042) with cholinesterase levels. This study concluded that the correlation between PPE usage showering and clothes with cholinesterase levels, also cholinesterase levels were negative correlated with GPx levels. The authors suggested that farmers are encouraged to be fully equipped with PPE and maintaining good personal hygiene to reduce pesticide exposure.**

***Keywords*— Cholinesterase, Free Radical, GPx, Oxidative stress, Pesticide.**

## I. INTRODUCTION

The use of chemical pesticides on the agricultural can lead to the inclusion of pesticides in the body through oral/ingestion, skin/dermal or inhalation/respiratory. Pesticides that enter the body will react with the cytochrome P450 by pulling 1 electron that causes the formation of ROS.[1] Glutathione peroxidase (GPx) is one of the enzymatic antioxidants that serve to protect the tissues from oxidative stress.[2] Research conducted on farmers to determine the exposure of pesticides against oxidative stress with GPx biomarkers in Turkish farming region, obtained the results that GPx level was significantly higher in farmer groups mixing Pesticides compared to farmers who did not mix pesticides. Research in the Spanish region also showed that there was a difference in Gpx activity with P-value of 0.001 among the spraying farmers exposed to high doses of pesticides with farmers exposed to low dose of pesticides. Research conducted in Egypt in 2011 concerning the assessment of toxicity and oxidative stress on farmers exposed to organophosphate and Carbamate, showed a negative correlation between cholinesterase and GPx levels indicating that the lower the ChE levels in the body, the higher the GPx activity in the body (P-value of 0.05).[3] Preliminary study conducted showed that farmers in Karanganyar Regency Area sprayed Pesticides 3 times per week by mixing 2 to 3 types of pesticides. This study aimed to analyse the historical relationship of pesticide exposure with GPx levels in onion farmers in Karanganyar Regency.

## II. METHODOLOGY

This study was cross-sectional analytical observational research. The population in the study amounted to 200 male and female farmers in the Kalisoro region of Karanganyar Regency. Sampling was carried out by a random sampling technique with the inclusion criteria of the male onion. Farmers who used chemical pesticides,

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aged less than 65 years, and did not suffer from degenerative diseases. The samples in this study amounted to 53 male farmer respondents. Research was conducted in Kalisoro village, Karanganyar Regency in July 2019. Primary data obtained through the examination of cholinesterase levels in Regional Health Laboratory (LABKESDA) of Semarang and GPx rate inspection conducted in GAKI Laboratory of Faculty of Medicine, Diponegoro University. Interviews conducted to identify the respondents' characteristics and history of pesticide exposure. Secondary data of agricultural data obtained from the Agriculture service of Karanganyar Regency and monographic data from the village of Kalisoro, District of Tawangmangu, Karanganyar Regency.

## III. RESULT

### 1) Respondents' characteristics

*Table 1. Respondents' characteristics*

| Variable (n = 53) | Mean (SD) | Median (Min-Max) | f | % |
|---|---|---|---|---|
| **Age** | 52,98 (10,48) | 55,00 (27-64) | | |
| **Weight (kg)** | 55,92 (8,04) | 55,00 (39-80) | | |
| **Height (cm)** | 157,38 (6,18) | 158,00 (142-175) | | |
| **Working period (years)** | 27 | 30 (2-55) | | |
| **Working period (years)** | | | | |
| > 20 years | | | 33 | 62,3 |
| ≤ 20 years | | | 20 | 37,7 |
| **Frequency of spraying** | | | | |
| Frequently (≥ twice a week) | | | 25 | 47,2 |
| Rarely (< twice a week) | | | 28 | 52,8 |
| **Working duration (hours)** | 6 (1,79) | 7 (2-10) | | |
| **Working duration (hours)** | | | | |
| > 40 hours per week | | | 43 | 81,1 |
| ≤ 40 hours per week | | | 10 | 18,9 |
| **Use of personal protective equipment** | | | | |
| Complete | | | 30 | 56,6 |
| Incomplete | | | 23 | 43,4 |
| **Mixing procedure** | | | | |
| Manually by hands | | | 0 | 0 |
| Using a stirrer | | | 53 | 100 |
| **Amount of mix** | | | | |
| > 2 types of pesticides | | | 29 | 54,7 |
| Single type | | | 24 | 45,3 |
| **Smoking** | | | | |
| Yes | | | 16 | 30,2 |
| No | | | 37 | 69,8 |
| **Consumption of medicine / herbs** | | | | |
| Yes | | | 33 | 62,3 |
| No | | | 20 | 37,7 |
| **Personal Hygiene** | | | | |
| **Shower habit** | | | | |
| No | | | 25 | 47,2 |
| Yes | | | 28 | 52,8 |
| **Cloth changing habit** | | | | |
| No | | | 15 | 28,3 |
| Yes | | | 38 | 71,7 |

The average age of respondents was 52.98 years old, the youngest respondent aged 27 years and the oldest respondent aged 64 years. Respondents had an average working time of 27 years, the shortest working period was 2 years and the longest was 55 years. A total of 25 respondents (47.2%) sprayed ≥2 times a week and 28 respondents (52.8%) sprayed less than 2 times a week. A total of 56.6% of respondents did not wear complete personal protective equipment. All respondents used the stirring tool when mixing pesticides.

Respondents who used more than 2 types of pesticides were 29 respondents (54.7%), while 24 respondents (45.3%) used single pesticides. Respondents who smoke were fewer than the respondents who did not smoke (16 [30.2%] vs 37 [69.8%]). Respondents who were routinely taking medication and herbs were 33 respondents (62.3%). Respondents who took a bath after spraying were more than 28 respondents (52.8%), while respondents who did not take a bath after spraying were

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25 respondents (47.2%). Also, respondents who changed clothes after spraying were more than 38 respondents (71.7%) while respondents who did not change clothes after spraying were 15 respondents (28.3%).

**2) The Laboratory Examination Results of Blood Cholinesterase and GPx**

*Table 2. The Laboratory Examination Results of Blood Cholinesterase and GPx*

| Variable | Mean | Min | Max | Normal Value |
|---|---|---|---|---|
| Cholinesterase (U/L) | 6404,85 | 4315 | 10862 | 4620-11500 |
| Glutathione peroxidase (ng/ml) | 14,1468 | 0,76 | 180,90 | 0,5-10 |

The average level of blood cholinesterase was 6404.85 U/L with a minimum value of 4315 U/L and a maximum value of 10862 U/L. The Center for Health Laboratory and Medical Equipment Testing of Central Java province set the normal rate for cholinesterase screening of 4620-11500 U/L. In the results of Glutathione peroxidase (GPx) level obtained an average of 14.1468 ng/ml, with a minimum value of 0.76 ng/ml and a maximum value of 180.90 ng/ml.

**3) The Relationship between Cholinesterase and Glutathione Peroxidase (GPx) Levels**

*Table 3. The Relationship between Cholinesterase and Glutathione Peroxidase (GPx)Levels*

| Variable | Glutathione peroxidase (GPx) | |
|---|---|---|
| | R | *p* |
| Cholinesterase | -0,310 | 0,024 |

There was a significant correlation between cholinesterases and glutathione peroxidase (GPx) levels. The correlation coefficient was -0.310, indicating that there was a low correlation between the cholinesterases and glutathione peroxidase (GPx) levels, and the negative correlation was counterclockwise, so the higher the cholinesterase levels, the lower Glutathione peroxidase (GPx) levels, likewise vice versa.

**4) The Relationship between Working Duration and Working Period with Cholinesterase Level**

*Table 4 The Relationship between working duration and working period with cholinesterase level*

| Variable | Cholinesterase | |
|---|---|---|
| | R | *p* |
| Working duration | 0,205 | 0,141 |
| Working period | -0,009 | 0,949 |

According to table 4, there was no significant correlation between the working duration and cholinesterase level and the correlation was counterclockwise, so the longer the working duration, the lower the cholinesterase level, and vice versa. Variable of working period was known to have no significant correlation with cholinesterase level. The correlation coefficient was unidirectional (r = -0.009), so the longer the working period, the higher the cholinesterase level, and vice versa.

**5) The Difference in Average Spraying Frequency, The Use of Personal Protective Equipment, Amount of Mix, and Personal Hygiene with Cholinesterase Level**

*Table 5. The Difference Result in Average Spraying Frequency, The Use of Personal Protective Equipment, Amount of Mix, and Personal Hygiene with Cholinesterase Level*

| ***Variable*** | ***Mean Rank*** | ***p*** |
|---|---|---|
| ***Frequency of spraying*** | | |
| *"Frequently" ≥ 2 times a week (n = 25)* | *27.22* | *0,922* |
| *"Rarely" < 2 times a week (n = 28)* | *26,80* | |
| ***The use of personal protective equipment*** | | *0,040* |
| *"Complete" (n = 30)* | *30,82* | |
| *"Incomplete" (n = 23)* | *22,02* | |
| ***Amount of mix*** | | |
| *"> 2 types of pesticides" (n = 32)* | *27,13* | *0,942* |
| *"Single pesticides" (n = 21)* | *23,81* | |
| ***Personal Hygiene*** | | |
| ***Cloth*** | | |
| *"Not changing the cloth after spraying" (n = 15)* | *20,30* | *0,047* |
| *"Changing the cloth after spraying" (n = 38)* | *29,64* | |
| ***Bath*** | | |
| *"Did not take a bath after spraying" (n = 29)* | *31,56* | *0,042* |
| *"Take a bath after spraying" (n = 24)* | *22,93* | |

Referring to the statistical test result of table 5 above, it was known that the p-value of 0.922 meant there was no difference in cholinesterase levels in farmer groups with the spraying frequency of more than 2 times a week or farmer groups with the spraying frequency of fewer than 2 times a week. In personal protective equipment application variable, it was known that the p-value of 0.040 meant there was a difference in cholinesterase levels in farmers group who used incomplete personal protective equipment with farmers group who used complete personal protective equipment. There was no difference in cholinesterase levels in farmer groups who used more than two types of pesticides as well as in farmer groups who used single pesticides (p = 0.942). In personal hygiene variable, clothes changing and bathing, p-value were 0.047 and 0.042, respectively which meant that there was a difference in average cholinesterase levels in farmer groups who did not take a bath after spraying with farmers group who took a bath after spraying. It also meant that there was a difference in average cholinesterase levels in farmer groups who did not change clothes after spraying with farmers groups who changed clothes after spraying.

## IV. DISCUSSION

Pesticide poisoning can lead to increased ROS. Pesticide of organophosphate and carbamate can not only inhibit AChE but also trigger the occurrence of ROS that cause oxidative stress. Reactive oxygen species (ROS) cause damage due to their very unstable and highly reactive nature to influence the changing structure of a cell.[4] GPx is one of the antioxidants that exist in the body that serves to break the ROS chain by converting $H_2O_2$ to $H_2O$ so that it does not form OH in the body and GPx activity can reduce more than 90% of hydrogen peroxide and 70% of organic peroxide.[5] Statistical test results in this study showed if levels of cholinesterase on farmers who were exposed to pesticides decreased, then levels of GPx got higher. This was in line with the study of Magda on 51 spraying farmers in Egypt stating that there was a significant correlation between the levels of the oxidative stress measured by GPx levels ($P = 0.05 < 0.05$, $R = -0.19$).[6]

The result of working duration variable was in line with the study Rustia in 2009 showing that there was no significant relationship between the length of work per day with the cholinesterase level (p = 0, 76).[7] The average duration of work is 7 hours. The longest spraying time is 42 hours per week and the shortest spraying time is 0.25 hours per week. The results of the study differed from the theory which explained that the duration of the work of pesticide spraying farmers was related to the amount of exposure to pesticides entering the body which had an impact on cholinesterase activity. The study of Agung in 2013 on onion farmers in Ngurensiti Pati showed that there was no relationship between the duration of work with cholinesterase level with P-Value of 1,000.[8] The growing number of farmers working mainly in spraying will affect the amount of exposure to pesticide into the body. According to Sungkawa (2008), the duration pesticides spraying should be less than 2 hours.[9] It is different from the Ministry of Health (2003) stating that the length of pesticides spraying per day should not be more than 5 hours and not more than 5 days per week.[7] Whereas according to WHO, the maximum limit of farmers at risk of pesticide poisoning is five hours per day/thirty hours per week.[10] Working duration of each farmer in the agricultural area can cause a difference in the amount of exposure to pesticides that accumulate in the body. Chronic poisoning can occur on farmers who are exposed to pesticides continuously within a certain period of time. The disturbance caused by pesticides with low kill power used in high rate setting is greater when compared with pesticides with high kill power but used in low rate setting.[10]

The farmers who have been exposed to pesticide for a long time are at risk of increased pesticide toxicity because the amount of pesticides entering the body will be higher. This research differs from the theory that explains that the longer a person is in the agricultural environment and in contact with pesticides, the higher the risk of exposure to pesticides so that it can affect the low levels of blood cholinesterase. According to Prabowo (2002), the symptoms of pesticide-induced poisoning will disappear within 24 hours, in 2 weeks the level of cholinesterase will return to normal in red blood cells while for cholinesterase in plasma takes 3 weeks to return to normal.[11] It is In line with the study of Budiawan (2013) which mentioned that there was no correlation between the cholinesterase levels with the working period of onion farmers (p-value = 1,000).[8] The same thing was delivered by Reni (2013) stating that there was no correlation between the working period of the farmers with the incidence of pesticide poisoning in onion farmers with a significance value of 0.324 and a value of 95% CI of 0,810 (0,699-0,937).[12] There is no relationship between a work period and cholinesterase levels in this study. It can be influenced by the geographical condition of the study area, where Kalisoro Village is a highland area that has a low temperature. Low-temperature environments can cause pesticides that have been sprayed not to evaporate and are inhaled by farmers

Pesticide poisoning can occur via oral, dermal or inhalation. Pesticides can enter the body through air and skin. Poisoning through the air can occur because of the wind, which causes the inspiration of the pesticides. Poisoning through the skin can occur when the farmer exposed to pesticides without wearing any personal protective equipment (e.g. glove). Other poisoning can occur when farmers sweat, which causes the absorption of pesticides into the body faster. Therefore, proper use of personal protective equipment during spraying can prevent pesticides from entering the body. The results of this study was similar to the study of Irnawati in 2005 showing that there was a difference between farmers who did not use complete personal protective equipment and farmers who used incomplete personal protective device with odds ratio of 5,3.[13] The study was also similar to the study of Agung in 2014 showing that there was a correlation between the use of personal protective equipment and cholinesterase levels with p-value of 0047.[8] PPE variable is related because the location of the farmer's house in Kalisoro is close to the agricultural area, and even the agricultural area is inside the farmer's house. The location of farmhouses close to agricultural areas can increase the potential for pesticide exposure.

The risk of poisoning was determined based on how often a person performed spraying (frequency of spraying).[14] Jeyaratnam and David (2010) suggested minimizing contact with pesticides of maximum 2 times a week.[15] The frequency of pesticide spraying had a close relationship with the incidence of pesticide poisoning characterized by decreased cholinesterase activity in the blood.[16] Absence of correlation between spraying frequency with cholinesterase levels could be caused by some farmers were entering the harvest period and will start planting new onions at the time of study conducted, so the frequency of spraying pesticides became less frequent. Also, while the study was taking place in the dry season, pesticide spraying was not done as much as in the rainy season, thereby affecting the level of pesticide poisoning in farmers. This was not in line with the study conducted by Rihardani in 2016 which showed that there was a significant correlation between the frequency of spraying with cholinesterase levels (P = 0.478). The lack of correlation in this study can be caused by differences in the number of pesticide mixtures used by farmers and the use of personal protective equipment. At the time, the research was being conducted in the dry season, so some farmers were starting to plant new onions, and some were entering the harvest period where spraying of pesticides was rarely done. The intensity of pesticide spraying can affect the level of pesticide poisoning, which results in decreased cholinesterase activity.

According to Priyanto (2007), farmers mixed 2-5 pesticide brands for one spraying. If two types of organophosphate insecticide mixed and administered concurrently, it will cause additive effect where the combined effect of the pesticide was equal to the amount of effect of each pesticide if given individually, in other words, the toxic effect of the pesticides were increasingly stronger.[17] This study was in line with the study done by Maria in 2009 in Ngablak Sub-district, showing that there was no correlation between the number of pesticides used with the incidence of pesticide poisoning (P-value = 1.00).[18] However, this was different from the study conducted by Okvitasari (2016) in Wonosobo Regency which mentioned that there was a correlation between the number of pesticides mixed with the incidence of pesticide poisoning (p-value = 0,011).[19] The difference in the relationship between the amount of pesticide mixture with cholinesterase levels in Kalisoro Village, Tawangmangu District, can be influenced by the behavior of farmers when mixing pesticides. As many as 100% of respondents (53 people) have used a mixer when mixing pesticides to minimize exposure to pesticides into the body through the skin.

The reduction of pesticides attached to the body will reduce absorption of fungicide into the body so that the binding of cholinesterases by pesticides can be reduced and the presence of symptoms and complaints due to pesticide poisoning is also reduced. If there is contact with pesticides then the clothes should be removed immediately, washed with running water, and take a bath immediately.[20] The results of this study were different from the study conducted by Reni in 2013 which indicated that there was no relationship between personal hygiene with the incidence of pesticide poisoning in onion farmers.[12] The study conducted by Budiono in 2004 showed that the proportion of pesticides poisoning to the body was 64.72% if the clothes used were not changed or washed after spraying.[20]

## V. CONCLUSION

The average level of farmer cholinesterase is 6404.85 U / L with a minimum value of 4315 U / L and a maximum value of 10862 U / L and a reference 4620-11500 U / L. 4 Farmers with abnormal cholinesterase levels (11.3%). Average levels of Glutathione peroxidase (GPx) 14.1468 ng / ml with a minimum value of 0.76 ng/ml and a maximum value of 180.90 ng/ml and reference 0.5-10 ng/ml. 6 Farmers who have abnormal cholinesterase (7.5%). There was no significant correlation between cholinesterase levels and the duration of the farmer's work (P = 0.141, r = 0.205). There was no significant correlation between cholinesterase levels and the farmer's working period (p = 0.949, r =-0.009). There was no difference in the average cholinesterase levels in the farmer group with the frequency of spraying of more than 2 times a week and in the farmer groups with the frequency of spraying of fewer than 2 times a week (P = 0.922). There was a difference in average cholinesterase levels in the group of farmers who used incomplete personal protective equipment with a group of farmers who used complete personal protective equipment (P = 0.040). There was no difference in cholinesterase levels in farmer groups who used more than 2 types of pesticides as well as in farmer groups who used single pesticides in one spraying (P = 0.942). There was a difference in average cholinesterase levels in farmer groups who did not take a bath after spraying with a group of farmers who took a bath after spraying (P = 0.047). There was a difference in average cholinesterase levels in farmer groups who did not change clothes after spraying with a group of farmers who changed clothes after spraying (p = 0.042).

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