



The Levels of Cholinesterase Enzyme and Hemoglobin in Linggasari Village's Farmers

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Article Info

Article History:

Submitted November 2023

Accepted January 2023

Published April 2023

Keywords:

cholinesterase enzyme,
hemoglobin, pesticide

DOI

<https://doi.org/10.15294/kemas.v18i4.40451>

Abstract

Farmers in Linggasari Village often mix pesticides, not using pesticides that follow the dose, not using PPE completely, eating, drinking, or smoking, did not clean themselves after spraying. Some types of pesticides can reduce cholinesterase enzyme and hemoglobin levels. The study aimed to analyze the factors correlated with the cholinesterase enzyme and hemoglobin levels of sprayer farmers. It was a cross-sectional study conducted from March to April 2022. The population of this study was all farmers who use pesticides. As many as 30 farmers qualify the inclusion criteria, namely male spraying farmers aged over 18 years, maximum last contact time with pesticides was two months before the study. The independent variables were age, BMI, working period, spraying frequency, duration of spraying, and PPE usage score. The dependent variables included cholinesterase and hemoglobin levels. Bivariate analysis was assessed with Pearson Correlation Test or Spearman Correlation Test, while multivariate analysis used linear regression. The results showed that age ($p=0,032$, $r=0,391$) and BMI ($p=0,036$, $r=0,385$) correlated with cholinesterase enzyme, and age ($p=0,000$, $r=0,615$) correlated with farmers' hemoglobin levels. The results of multivariate analysis, the most influential factor on cholinesterase enzyme and hemoglobin levels was age.

Introduction

Pesticides are widely used in the agricultural sector to eradicate pests so that the quality of agricultural production can be abundant and good quality. However, if pesticides are used unwisely, there will be negative impacts on the environment, farmers' health, and public health in general. Exposure to pesticides in preparing equipment, mixing pesticides, spraying, cleaning tools and work clothes, cleaning grass and pests, watering plants, and harvesting can cause poisoning. The global pesticide poisoning rate reached the highest value of 385 million cases, with an estimated death of 11,000 per year (Boedeker et al., 2020).

The National Poisoning Information

Center (2016) noted that the incidence of pesticide poisoning in Indonesia was 771 cases and is expected to increase annually. The incidence of pesticide poisoning in several areas in Indonesia is very high. Based on monitoring of the cholinesterase enzyme in 347 farm workers in Central Java, 23.64% of workers were moderately poisoned, while 35.73 were severely poisoned (Anam et al., 2015).

The community of Linggasari Village, Kembaran District, Banyumas Regency work as farmers with as many as 489 people (7.9%) and is the second largest group of workers after casual daily workers, namely 1,116 people (18.13%). It is supported by an area of 235 hectares of lowland rice and 182 hectares of corn. The use of pesticides by farmers in

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Linggasari varies widely, including carbamates, pyrethroids, paraquat, organochlorines, and organophosphates. Farmers use pesticides according to pest attack, where the number of pesticides used by farmers ranges from 2-3 types during one planting period. However, in its use, farmers often mix pesticides, not following the dose, do not use Personal Protective Equipment (PPE), and unsafe behavior after spraying, such as eating, drinking, and smoking without cleaning themselves.

Organophosphates act as cholinesterase inhibitors so that they can reduce cholinesterase levels in the blood. Organophosphate pesticides can also reduce the number of erythrocytes, hemoglobin levels, leukocyte counts, and lymphocytes (Shamoushaki, 2012). Excessive exposure to pesticides can interfere with the nervous system cancers, diabetes, and reproductive disorders (Mostafalou and Abdollahi, 2013), and the effect on children's IQ impaired (Suwondo et al., 2021). Although pesticide exposure and its effect on health, studied in several areas in Indonesia, no research has been conducted on the negative impact of pesticide use in Linggasari Village, Kembaran District, Banyumas Regency. The study aimed to analyze the factors that correlated with levels of the enzyme cholinesterase and hemoglobin of spraying farmers in Linggasari, Kembaran, Banyumas Regency.

Method

This study was a cross-sectional study, aiming to analyze the factors correlated with the levels of cholinesterase enzymes and hemoglobin in spraying farmers in Linggasari, Kembaran, Banyumas Regency. As many as 30 farmers qualify the inclusion criteria, i.e., males, aged over 18 years, who sprayed using synthetic pesticides, the last contact time with pesticides was a maximum of two months before the

study. The independent variables were age, BMI, working period, spraying frequency, duration of spraying, and PPE usage score. The dependent variables in this study were levels of the enzyme cholinesterase and hemoglobin.

Data collection includes respondent characteristics, history of pesticide exposure, and use of PPE carried out through interviews. BMI was calculated based on a standardized formula i.e., body weight (in Kg) divided by height (in meters) squared. Cholinesterase and hemoglobin levels were measured by taking 3 ccs of venous blood samples. Then the blood samples were taken to the Prodia Purwokerto Laboratory. The cholinesterase enzyme was measured by the kinetic photometric method using the semi-auto chemistry analyzer model Biolyzer 100. Hemoglobin was measured using the SLS method Hemoglobin with an automated hematology analyzer. Data analysis was by univariate, bivariate, and multivariate analysis. Bivariate analysis was assessed using the Pearson Correlation Test or the Spearman Correlation Test. Multivariate analysis to find out which factors have the most influence on the cholinesterase enzyme and hemoglobin levels.

Result and Discussion

Linggasari Village is one of the villages that has a relatively large area compared to other villages in the Kembaran District. It is about 239.811 hectares. The area covers an area of technically irrigated land of approximately 0.70 hectares, rainfed rice fields covering an area of 167.688 hectares, dry land or settlements covering an area of 60 hectares, land for public facilities covering an area of 7.20 hectares, a field of 1 hectare, village government offices covering an area of 0.70 hectares. Roads and rivers are around 5.513 hectares (Mutahir, 2021).

TABLE 1. Results of Univariate Analysis on Spraying Farmers in Linggasari

Variables	Mean \pm Standard Deviation	Minimum-Maximum	Unit
Age	61.93 \pm 9.798	36-84	year
BMI	22.41 \pm 2.991	17.79-28.58	-
Working Period	32.17 \pm 15.976	3-57	year
Frequency of Spraying	4.67 \pm 5.175	1-30	per month
Duration of Spraying	2.617 \pm 1.986	0.5-10	hour
PPE Usage Score	15.67 \pm 4.894	6-24	-
Cholinesterase Enzyme Levels	9,850 \pm 2,142	6,738-15,319	U/L
Hemoglobin Levels	14.6 \pm 1.1	11.9-16.5	g/dL

Source: Primary Data, 2022

Table 1 showed the youngest respondent was 36 years old, the oldest was 84 years old, and the average age was 61.93. Productive age is in the range of 18-54 years. The results indicate that the average age of the respondents was elderly. The minimum BMI was 17.79, and the maximum was 28.58, with an average of 22.41, so most respondents' BMI was normal. Respondents in this study had the lowest working period of 3 years and the longest of 57 years, with an average was 32.17 years. The lowest frequency in this study was one spray per month, and the highest frequency was 30 sprays per month, with an average was 4.67 per month. The minimum duration of spraying was 0.5 hours, and the maximum was 10 hours, with an average was 2.617 hours. The minimum PPE usage score was 6, and the maximum was 24, with an average was 15.67. The minimum

cholinesterase enzyme level was 6.738, and the maximum was 15.319, with an average was 9.850 U/L.

The results of laboratory examinations showed that 28 people (93,33%) had cholinesterase levels in the normal category, namely at levels of 6.738 – 12.887 U/L where the normal value was 4.260 – 11.250 U/L for age < 40 years and 5.320 – 12.920 U/L for age > 40 years. In this study, two people (6,67%) with high cholinesterase levels above 12,920 U/L. The average hemoglobin level was 14.6, with a minimum of 11.9 and a maximum of 16.5 g/dL. Anemia in women if the hemoglobin levels are less than 12,0 g/dL, while in men, less than 13,0 g/dL (Cappellini and Motta, 2015). The results showed that 4 people (13,33%) experiencing anemia.

TABLE 2. Results of Bivariate Analysis of Age, BMI, Working Period, Frequency of Spraying, Duration of Spraying, and PPE Usage Score with Cholinesterase Enzyme and Hemoglobin Levels.

Variables	Correlation coefficient (Cholinesterase Enzyme)		Correlation coefficient (Hemoglobin)	
	p	r	p	r
Age	0,032	-0,391	0,000	-0,615
BMI	0,036	0,385	0,054	0,355
Working Period	0,673	-0,080	0,470	-0,137
Frequency of Spraying	0,756	-0,059	0,579	-0,106
Duration of Spraying	0,299	0,196	0,261	-0,212
PPE Usage Score	0.703	0.073	0,179	0,252

Source: Primary Data, 2022

There was a significant correlation between age and cholinesterase levels (p-value=0.032). The Pearson correlation value was 0.391, which indicates a weak correlation. The correlation showed a negative sign, meaning

that as the age increases, the respondent's cholinesterase levels decrease. This study is in line with the study in East Kalimantan (Ramdan, Candra and Purwanto, 2020). Older age experiences cumulative pesticide exposure,

which can affect farmers' health. Health risks increase in older farmers with time of exposure and aging. Older age is also associated with neurological deficits (Moza et al., 2021). With increasing age, especially at age ≥ 65 years, cholinesterase levels decrease (Matsuo and Tazawa, 2019). The results of this study indicate that elderly tend to experience a decrease in the cholinesterase enzyme, so they are very susceptible to pesticide poisoning. So farmers should be more aware of decreasing levels of cholinesterase enzymes caused by exposure to pesticides, especially with increasing age.

There was a significant correlation between age and hemoglobin levels of spraying farmers ($p\text{-value}=0,000$). A Pearson correlation value of 0.615 indicates a strong correlation with the direction of the correlation is negative, which means that the older the respondent's hemoglobin level decreases. In this study, all respondents who experienced anemia were elderly. They are at risk for anemia due to physical, psychological, physiological, functional, and metabolic changes (Lopes et al., 2022). Anemia in elderly can be caused by nutritional problems such as malnutrition which is caused by anorexia, lack of appetite, difficulty chewing or swallowing, dental and mouth problems, and nausea (Braz, Duarte and Corona, 2019).

Other common causes of anemia in the elderly are chronic kidney disease, chronic inflammation, and blood loss from the gastrointestinal (GI) tract due to GI pathology (Lanier, Park and Callahan, 2018), parasitic infections, hemoglobinopathies, and lead poisoning (Shaw and Friedman, 2011). Other factors also influence the incidence of anemia is education. The education of respondents who have anemia was elementary school. Kim and Son (2019) stated that patients who graduated from elementary school only or had no education were more likely to experience anemia than those who graduated from junior high school or higher (Kim and Son, 2019). In the elderly, anemia is a risk factor for cardiovascular disease, fatigue, reduced cognitive function, physical function, and quality of life, and indicates vulnerability (Onem et al., 2010). Neurologic complications due to anemia cause a decrease in physical

ability, which results in falling easily (Gabrilove, 2005).

There was a significant correlation between BMI and cholinesterase levels ($p\text{-value} = 0.036$). A Pearson correlation value of 0.385 which indicates a weak correlation. The correlation showed a positive sign, which means that the higher the BMI, the higher the level of the cholinesterase enzyme. BMI was significantly associated with organophosphate poisoning. Obese patients who experience poisoning require prolonged hospital stays (Jung et al., 2014). Organophosphates are lipophilic. Therefore they are thought to have a large volume of distribution and are rapidly distributed into tissues and fat.

This study is per a study in China (Han et al., 2019) and Saudi Arabia (Hamouda et al., 2019). Over-nutritional status causes pesticides to be stored in body fat as residue, so they can be at risk of acute and chronic pesticide poisoning. Poor nutritional status causes a decrease in body resistance and protein so that it is susceptible to infection and disturbances in the formation of cholinesterase enzymes (Kurniasih et al., 2013). Thus, farmers should keep their BMI within the normal range to prevent a decrease in the cholinesterase enzyme.

There was no significant correlation between BMI and hemoglobin levels of respondents ($p\text{-value} = 0.054$). Pearson correlation value was 0.355, which showed a weak correlation, the direction of the correlation was positive, which means the higher the BMI, the higher the hemoglobin levels. Poor nutritional status causes low hemoglobin levels and lowers body resistance and susceptibility to infection, so people with poor nutritional status are susceptible to disease or poisoning. Poor nutritional conditions cause the protein in the body to be very limited, so it interferes with the formation of the cholinesterase enzyme (Kurniasih, Setiani and Nugraheni, 2013).

The working period is the number of years a person has worked as a farmer. There was no significant correlation between the working period and levels of the cholinesterase enzyme ($p\text{-value} = 0.673$). The Pearson correlation value was 0.080, which indicated a very weak correlation. The correlation showed a negative sign, which means that the longer the working

period, the lower the level of the cholinesterase enzyme. In this study, farmers sprayed in the morning or evening, which is the optimal time for spraying. The best spraying time is in the morning before 11.00 and evening after 15.00. Spraying during the day causes the pesticides to evaporate and decompose, thereby increasing farmers' exposure to pesticides by inhalation or through the skin which can cause pesticide poisoning (Suparti, Anies and Setiani, 2016). However, it is necessary to be aware that the longer the working period as a farmer, the more exposed to pesticides, so the greater the risk of poisoning. There was no significant correlation between the working period and hemoglobin levels of respondents (p -value=0,470), with a weak correlation (r =0,137), and the direction of the correlation was negative, which means the higher the working period, the lower the hemoglobin level.

The length of the working period is related to the risk of pesticide poisoning, which can cause low hemoglobin levels. In this study, low hemoglobin levels in farmers can be caused by other factors. Factors affecting hemoglobin levels include age, gender, race, nutritional and environmental factors, altitude, smoking habits, drugs consumed, and parasitic infections. In addition, there is no correlation between working period and hemoglobin levels. It could be because farmers have applied the practice of spraying according to the direction of the wind. Farmers who spray against the wind will be more exposed to pesticides, so poisoning is easier to occur.

The frequency of spraying is the number of days of spraying during the last month. There was no significant correlation between the frequency of spraying and levels of the cholinesterase enzyme (p -value = 0.756). The correlation value was 0.059, which indicated a very weak correlation. The correlation showed a negative sign, meaning that the more frequency of spraying, the lower the level of the cholinesterase enzyme. This study was different from research in Gawu-Gawu Bouso Village North Gunungsitoli Sub-District, Gunungsitoli City (p -value=0,012) (Hotang, Ashar and Hasan, 2020).

The frequency of spraying increases the risk of exposure to pesticides. In this study, the

average respondent sprayed was 4 times a month. These conditions can allow for an increase in the cholinesterase enzyme. A one week's rest, minimum, can increase cholinesterase activity in the blood of farmers. Bioaccumulation of pesticide residues due to frequent spraying has the potential to reduce hemoglobin levels. Spearman test results obtained p value = 0,579, which means no significant correlation between frequency of spraying and farmers' hemoglobin levels. A correlation value of 0.106 indicated a weak correlation, while the direction of the correlation was negative, meaning that the more frequency of spraying, the lower the respondent's hemoglobin level. Infrequent contact can reduce the risk of anemia in farmers. Other factors that may affect the hemoglobin level of the respondent include the characteristics of the respondent and adequate rest, which causes the farmer to have a good hemoglobin level.

There was no significant correlation between the duration of spraying and levels of the cholinesterase enzyme (p -value=0,299). The Spearman correlation value was 0.196, which indicated a very weak correlation. The direction of the positive correlation means the longer the duration of spraying, the higher the level of the cholinesterase enzyme. This result can be because the average farmer uses pesticides for less than 5 hours. Based on WHO, the duration is five hours per day or thirty hours per week (Yushananta, Ahianti and Anggraini, 2020). The duration of spraying is related to the amount of time in contact with pesticides. The longer the spraying, the more contact time with pesticides increases. The risk of poisoning and the accumulation of pesticides is also increasing. Madaan's research showed lower cholinesterase in spray workers compared to non-spray farmers.

To restore the activity of the cholinesterase enzyme, farmers can rest first after spraying. Plasma cholinesterase levels will return to normal within 3 weeks, while blood cholinesterase enzymes will take 2 weeks without exposure. Although the acute effect of acetylcholinesterase enzyme activity is only temporary and usually only lasts 1-2 weeks, acute pesticide poisoning can cause neurological disorders in the long term due to the inhibition

of the cholinesterase enzyme continuously (Samosir, Setiani and Nurjazuli, 2017). There was no significant correlation between spraying time and farmers' hemoglobin levels (p -value = 0.261). The correlation value of 0.212 indicated a weak correlation, the direction of the correlation was negative, which means that the longer the spraying duration, the lower the respondent's hemoglobin level. The duration of spraying is related to the length of work that allows farmers to come into contact with pesticides. In this study, the average farmer sprayed for 2,617 hours, so it was still within a safe time limit.

PPE can prevent pesticides enter the body so that it can avoid poisoning. In this study, the use of PPE was outlined in the form of scoring. The more complete the PPE used, the higher the score. The results showed that the average PPE usage score, minimum and maximum score, respectively, was 15.67, 6 and 24. There was no significant correlation between the use of PPE and cholinesterase levels (p -value = 0.703. Pearson's value of 0.073 indicated a very weak correlation, and the direction of the correlation was positive, indicating that the higher the APD score, the higher the level of the cholinesterase enzyme.

PPE for spraying farmers are masks, hats, goggles, gloves, boots, and aprons to avoid exposure to pesticides on the skin and body (Damalas and Abdollahzadeh, 2016). The routes of entry of pesticides are through the skin, mouth, inhalation and eyes. Skin exposure is affected by pesticide residues, pesticide formulation, amount, and duration of exposure, presence of other substances on the skin, temperature, humidity, and use of PPE (Kim, Kabir and Jahan, 2017). Oral exposure usually occurs accidentally, for example, with carelessness or intentional reason. Farmers can get poisoned by not washing their hands before eating or smoking after spraying. The potential exposure to pesticides through inhalation is due to volatile substances in pesticides. Pesticides can also enter through the eyes due to not wearing glasses or face shields.

There was no significant relationship between the PPE usage score and the hemoglobin levels of respondents (p -value = 0.179). Pearson correlation value was 0.252,

which showed a weak correlation, the direction of the correlation was positive, which means the higher the PPE usage score, the higher the hemoglobin level. In red blood cells, pesticides can form sulfhemoglobin and methemoglobin, which causes hemoglobin levels to decrease. The sulfur content in pesticides causes the formation of sulfhemoglobin, while methemoglobin is formed due to oxidized iron or nitrite bonds with hemoglobin (Yushananta, Ahyanti and Anggraini, 2020). Reducing health risks due to exposure to pesticides can be done by applying the correct spraying practices, namely by paying attention to the spraying time and wind direction. The best time for spraying is in the morning or evening because the temperature is lower than in the afternoon. Pesticides evaporate quickly at temperatures above 30°C, so farmers are advised not to use them during the afternoon (Kim, Kabir and Jahan, 2017). The best spraying practice is in the same direction as the wind.

Multivariate analysis was carried out on factors with a p -value ≤ 0.25 from the bivariate analysis. The results of multivariate analysis using the backward linear regression method showed that the most influential factor on cholinesterase enzyme and hemoglobin levels in farmers was age with a p -value and correlation value respectively (p -value = 0.032, r 0,391), (p -value=0,000, r =0,615). The results of the multivariate analysis can be concluded, as age increases, the levels of cholinesterase enzymes and hemoglobin decrease. It can be attributed to the fact that in old age, there has been a decrease in body function due to degeneration, nutritional problems, and psychological problems. This decrease has made elderly farmers very vulnerable to pesticide poisoning and anemia. Such conditions can also affect the quality of life of farmers. Research showed that elderly tobacco farmers who were still active in agricultural activities experienced a decline in health and quality of life (Susanto and Widayati, 2018). In addition, the results of other studies showed that farmers in older age were significantly related to the risk of injury (Heaton et al., 2012). Therefore, it is necessary to have special treatment or prevention for elderly farmers to increase their safety and quality of life.

Conclusion

Based on the bivariate analysis, there was a significant correlation between age and BMI with cholinesterase enzyme levels in Linggasari Village farmers. There was no significant correlation between working period, frequency of spraying, duration of spraying, PPE usage score, and levels of cholinesterase enzyme in Linggasari Village farmers. The result of multivariate analysis, the most influential factor on cholinesterase enzyme was age. There was a significant correlation between age with hemoglobin levels. There was no significant correlation between BMI, working period, frequency of spraying, duration of spraying, PPE usage score, and hemoglobin levels in farmers. The result of multivariate analysis, the most influential factor on hemoglobin level was age.

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