Pesticide Exposure, Behavior of Farmer, and Activity of Cholinesterase Enzyme in Blood of Fertile Women Farmers

Pajanan Pestsida, Perilaku Petani, dan Aktivitas Enzim dalam Darah Petani Perempuan Usia Subur

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Abstract
Fertile women farmers are risky of suffering decrease of cholinesterase activity due to pesticide exposure. This study aimed to analyze relation between pesticide exposure and the exposure agent to cholinesterase activity of fertile women farmers at Kedunguter Village. This study used cross-sectional design on 94 fertile women farmers in 2015. Data was collected by observation, interview and cholinesterase test. Data analysis used chi-square test and analysis results showed a significant relation between pesticide types, working time, the use of gloves, hand-washing behavior to cholinesterase activity of fertile women farmers. Analysis results of this study showed that variable working time had the highest odds ratio (OR) score (OR = 14.072), so the variable working time is the most dominant variable in influencing cholinesterase enzyme. This study suggests that fertile women farmers should work not more than six hours per day.

Keywords: Behavior, cholinesterase enzyme, pesticide exposure

Introduction
Brebes District is the largest red onion-producing region in Central Java.1 The higher production of red onion, the more pesticides used in Brebes District. Pesticides most commonly used in Brebes District are organophosphate and carbamate insecticides.2 Organophosphates and carbamates are substance that may inhibit or inactivate cholinesterase enzyme activity.3

Health agency has conducted an observation of cholinesterase enzyme activity in blood of onion farmers in Brebes District every year. Results of the observation within the last five years showed that proportion of the highest incidence of cholinesterase disorder occured in 2012 was 56%. The observation in 2012 was conducted in 10 villages over Brebes District and the observation results showed that Kedunguter Village in Brebes Subdistrict was the village with the highest incidence of cholinesterase enzyme disorder worth 82%. Therefore, Kedunguter Village was selected as the location for this study.4

According to the prior study, any disorder/decrease of cholinesterase enzyme activity was found among fertile...
women around farming area. Fertile women are a vulnerable group due to pesticide exposure, considering effect of pesticides is substance that may influence establishment and growth of embryo. This statement is supported by the prior study conducted in Poland. Based on that study, there was a significant relation between pesticide exposure among mothers and incidence of low birthweight (LBW) and premature pregnancy. Disorder on pregnancy process may cause harm effects for child growth. Based on Government Regulation (Peraturan Pemerintah) 66 Article 44 of The Year 2014, one of actions to protect people from pesticide dangers is analyzing harmful risks of pesticide exposure impacts. Risk analysis can be conducted by measuring cholinesterase enzyme activity of fertile women farmers.

This study is not the first study conducted among fertile women at work scope of Brebes District Health Agency. The similar kind of study was already conducted by several researchers who observed relation between fertile women living in farming area and cholinesterase enzyme activity. Differ from previous studies, all respondents in this study were fertile women working as farmers, so all the respondents in this study were a risky group and observation was conducted in obtaining information concerning behavior of fertile women farmers. This study aimed to analyze relation between pesticide exposure and behavior of the exposure agent to cholinesterase enzyme activity of fertile women farmers and to determine dominant factors influencing the cholinesterase enzyme activity of fertile women farmers at Kedunguter Village, Brebes Subdistrict, Brebes District in 2015.

**Method**

This study used cross-sectional design. Cross-sectional design is study in aim to observe relation between risk factors and effects at the same time. Location of study was at Kedunguter Village, Brebes Subdistrict, Brebes District. This study was conducted on February 20th – May 20th 2015. Population was all fertile women working as active farmers because they always did red onion farming activity at field, but did not do pesticide spraying activity and they lived at Kedunguter Village, Brebes Subdistrict, Brebes District.

Sample of this study was as many as to 94 respondents obtained by using proportion estimating formula. Samples were fertile women at Kedunguter Village, Brebes Subdistrict, Brebes District selected by using random sampling method. Data was received from direct observation in the location of study namely interviewing respondents as conducted within one week before cholinesterase enzyme examined. The examination of cholinesterase enzyme activity that used Tintometer Kit Lovibond device was conducted by Brebes District Health Agency with doctors around the nearest primary health care while this study was being conducted, or with laboratory officer appointed by Brebes District Health Agency. Results of any relation found in this study was assessed by considering results of p value, odds ratio (OR) and confidence interval (CI).

**Results**

**Cholinesterase Enzyme Activity**

Decrease of cholinesterase enzyme activity was the impact emerged by excessive pesticide exposure. Being fertile woman farmer is such a work vulnerable to pesticide exposure. Table 1 presented data of results of cholinesterase test conducted among 94 fertile women farmers at Kedunguter Village in 2015.

In this study, 100% of fertile women farmers at Kedunguter Village had normal cholinesterase enzyme activity because of not having cholinesterase enzyme activity ≤ 75% based on cut off point. Therefore, it need determination of new cut off point in aim to ease statistical analysis. Before determining the new cut off point, data normality test (Kolmogorov-Smirnov) was early conducted to ensure normality of data distribution.

Results of data normality test on cholinesterase enzyme activity showed p value < α (0.05), so the distribution form was abnormal. Based on this weakness, a new cut off point was determined based on statistical approach by using quartile value. The middle value of the first quartile would be used as the new cut off point for cholinesterase activity in blood of fertile women farmers because the first quartile value was the value closest to the threshold of pesticide poisoning level. Table 2 presented category of cholinesterase activity based on result of cut off point. Table 2 showed most fertile women farmers who had activity > 87.5.

**Table 1. Results of Cholinesterase Enzyme Activity Test and Data Normality Test on Cholinesterase Enzyme Activity of Fertile Women Farmers**

| Cholinesterase Enzyme Activity | n | % | Kolmogorov-Smirnov Statistic | Df | p value | Q1 | Q2 | Q3 |
|-------------------------------|---|---|-----------------------------|----|--------|----|----|----|
| 75%                           | 6 | 6.4| 0.372                      | 94 | 0.000  | 87.5| 100| 100|
| 87.5%                         | 32| 34.0|                            |    |        |    |    |    |
| 100%                          | 56| 59.6|                            |    |        |    |    |    |
| **Total**                     | 94| 100.0|                            |    |        |    |    |    |

**Table 2. Distribution of Frequency of Cholinesterase Enzyme Activity in Fertile Women Farmers**

| Category of Cholinesterase Enzyme Activity of Fertile Women Farmers | n | % |
|---------------------------------------------------------------------|---|---|
| > 87.5                                                              | 38| 40.4|
| ≤ 87.5                                                             | 56| 59.6|
| **Total**                                                           | 94| 100|
Pesticide Exposure

Pesticide exposure got by fertile women farmers was the result from pesticide spraying activity done by men farmers. Fertile women farmers were active farmers who always did farming every day, but did not do pesticide spraying. The pesticide spraying activity was only performed by men farmers, so information regarding pesticide types was obtained from the men farmers. The information regarding pesticide types was obtained from observation conducted among 82 men farmers (pesticide spraying agent). Men farmers always used many types of pesticides and mixed two to three types of pesticides in spraying. Table 3 presented results of observation concerning pesticide types used by men farmers.

The information regarding types of active materials on Table 3 showed category of pesticide types received by fertile women farmers from pesticide types used by the spraying agent. Type of pesticide exposure among fertile women farmers was determined by considering the nearest location of the spraying agent with fertile women farmers while the observation conducted.

Table 4 presented relations of independent variables (pesticide exposure and the behavior of the exposing agent) and dependent variable (cholinesterase activity). Data presented on Table 4 showed a significant relation between pesticide types, working time, the use of gloves and hand-washing behavior to cholinesterase enzyme activity of fertile women farmers according to OR score.

Final model of this study showed that working time was the dominant factor influencing cholinesterase enzyme activity with OR score = 14.072 (Table 5).

Discussion

Results of cholinesterase enzyme activity test on fertile women farmers at Kedunguter Village showed 100% normal because the spraying farmers used more pesticide materials not from organophosphate category. Organophosphate and carbamate entering to the body tend to be bounded with cholinesterase enzyme that caused inactivation of cholinesterase enzyme. Such matter caused acetylcholine piled on nerve and occurrence of overstimulus on receptor nerve. Most spraying farmers used organophosphate pesticide only for additional mixing material, so the dose used was little. Although

| Type                  | Active Materials | n  | %    |
|-----------------------|------------------|----|------|
| Organophosphate       |                   |    |      |
| Profenofos            | 31               | 42 |
| Phenthoate            | 25               | 32 |
| Chlorpyrifos          | 19               | 26 |
| Total                 | 73               | 100|
| Non-organophosphate   |                   |    |      |
| Carbosulfan           | 23               | 17 |
| Cypermethrin          | 30               | 22 |
| Carbosulfan           | 23               | 17 |
| Chlorfenapyr          | 33               | 25 |
| Lufenuron             | 25               | 19 |
| Total                 | 154              | 100|

Table 3. Frequency of Types of Active Materials Used as Pesticide Mixture by 82 Farmers Spraying Pesticides

| Variable                      | Category                      | n  | %    | N  | %    | N  | %    | p value |
|-------------------------------|-------------------------------|----|------|----|------|----|------|---------|
| Cholinesterase Activity       | ≤ 87.5%                       |    |      | > 87.5% | Total | OR (95%CI) | p value |
|                               | n  | %    | N  | %    | N  | %    |         |
| Pesticide exposure            |     |      |    |      |    |      |         |
| Pesticide types               | Organophosphate               | 28 | 47.5| 31 | 52.5| 59 | 100 | 2.258   | 0.113   |
|                               | Non-organophosphate           | 10 | 28.6| 25 | 71.4| 35 | 100 | (0.924 - 5.520) | 1.000   |
| Working period                | > 13.5 years                  | 19 | 40.4| 28 | 59.6| 47 | 100 | (0.433 - 2.279) | 0.000   |
|                               | ≤ 13.5 years                  | 19 | 40.4| 28 | 59.6| 47 | 100 |         |         |
| Working time                  | > 6 hours/day                 | 21 | 80.8| 5  | 19.2| 26 | 100 | 12.6    | 0.000   |
|                               | ≤ 6 hours/day                 | 17 | 25.0| 31 | 75.4| 68 | 100 | (4.115 - 38.585) |         |
| Behavior of exposure act      | Use of mask                   | 37 | 41.6| 52 | 58.4| 89 | 100 | 2.846   | 0.645   |
|                               | Using mask                    | 1  | 20  | 4  | 80  | 3  | 100 | (0.306 - 26.308) |         |
|                               | Wearing long sleeve shirt     | 2  | 100 | 0  | 0   | 2  | 100 | 0       | 0.161   |
|                               | Not wearing long sleeve shirt | 36 | 39.1| 56 | 60.9| 92 | 100 | (0.40)  |         |
| Use of gloves                 | Not using gloves              | 13 | 5605| 10 | 43.5| 23 | 100 | 2.392   | 0.117   |
|                               | Using gloves                  | 25 | 35.2| 46 | 64.8| 71 | 100 | (0.918 - 6.232) |         |
| Hand-washing behavior         | Not washing hand before eating| 34 | 46.6| 39 | 53.4| 73 | 100 | 3.705   | 0.026   |
|                               | Washing hand before eating    | 4  | 19  | 17 | 82  | 21 | 100 | (1.136 - 12.086) |         |
| Bathing behavior              | Not immediately bathing and dressing up after going home from rice field | 2  | 66.7| 1  | 33.3| 5  | 100 | 3.056   | 0.565   |
|                               | Immediately bathing and dressing up after going home from rice field | 36 | 39.6| 55 | 60.4| 91 | 100 | (0.267 - 54.950) |         |
| Total                         | 38                           | 40.4| 56 | 59.6| 94 | 100 |         |
organophosphate was only used as additional mixing material while spraying pesticides, some fertile women farmers were found suffering the decrease of cholinesterase enzyme activity. There were 38 fertile women farmers who had cholinesterase enzyme activity ≤ 87.5%.

Organophosphate is the main agent in the decrease of cholinesterase enzyme activity.\textsuperscript{10,11} Bivariate analysis showed significant relation between pesticide type used by the spraying farmers and cholinesterase enzyme according to OR score. Meanwhile, multivariate analysis showed that pesticide type used by farmers would contribute to cholinesterase enzyme activity. Result of this study was aligned with previous study obtaining relation between pesticide types used by farmers and cholinesterase enzyme activity.\textsuperscript{12} Information about pesticide types was obtained by conducting observation among men farmers, land owners, or husbands of fertile women farmers who were spraying pesticides. After the information was obtained, such information would be categorized based on active materials and the pesticide types.

The longer a person worked by using pesticides, it would be aligned with effects of the decrease of cholinesterase enzyme activity.\textsuperscript{12} However, such case was not found in this study. Based on bivariate analysis results, no significant relation was found between working period and cholinesterase enzyme activity. Result of this study was aligned with previous study which did not find significant relation between working period and cholinesterase enzyme activity.\textsuperscript{13-15} According to bivariate analysis, 50% of fertile women farmers had working period ≥ 15.5 years, but this did not give any influence to farmer’s cholinesterase enzyme activity due to little dose got by fertile women farmers. Based on their activities, fertile women farmers would get pesticide exposure from irrigation water channel in rice field and pesticide residue on plants. Study conducted by Taufik & Yosmaniar,\textsuperscript{16} showed that Brebes District irrigation channel and plantation was polluted by organophosphate pesticide residue worth 2.7 – 3.2 g/L.

The longer a person in contact with pesticides every day, the more decreasing cholinesterase activity would be. Therefore, the maximum threshold to contact with pesticides should be no more than five hours per day, even Environmental Protection Agency (EPA) has smaller maximum threshold that is four hours per day to contact with pesticides.\textsuperscript{17,18} Results of bivariate analysis showed significant relation between farmer’s working time per day and cholinesterase enzyme activity, meanwhile multivariate analysis showed that working time was the dominant factor in influencing cholinesterase enzyme activity in blood of fertile women farmers. This was in accordance with the previous study that found out significant relation between working time and cholinesterase enzyme activity in blood.\textsuperscript{19}

This study found out fertile women farmers who worked more than six hours every day. They did additional activities, such as watering plants and harvesting. Based on reference, ability of organophosphate to enter and start influencing cholinesterase enzyme may occur in relatively short time.\textsuperscript{11} So that, from the analysis results of this study, fertile women farmers are hoped not to do farming activities more than six hours every day. It will definitely decrease productivity of fertile women farmers, so to maintain the productivity, fertile women farmers can do any other additional activities, such as participating at entrepreneurship activities that can maintain and increase their productivity. Therefore, government should provide any entrepreneurship training for fertile women farmers to avoid quite long pesticide exposure effects.

The use of mask takes an important role inhibiting the entering of pesticides to human’s body through inhalation.\textsuperscript{20} Disobedience to the use of self-protector (alat pelindung diri/APD) significantly related to cholinesterase on farmers.\textsuperscript{21} However, this study found out different results. Based on bivariate analysis, OR score = 2.846 (0.506 – 26.508). Because OR = 0.506 – 26.508 passed 1, this showed that no significant relation was found between the use of nose and mouth-covering mask and cholinesterase enzyme activity. Pesticides would be easier to be inhaled by fertile women farmers who did not use such mask while working. The pesticides inhaled by farmers can directly enter to blood circulation system in short time namely in second to minute, so it is regrettable if there are still many fertile women farmers who never use mask while working at the field.\textsuperscript{11} Most fertile women farmers do not use nose-covering mask. Therefore, fertile women farmers always feel such tightness while using the mask. Brebes District Health Agency should hold any counseling and grant operational assistance to fertile women farmers in providing masks worthy used during farming activities.

Fertile women farmers should wear long sleeve shirt to avoid direct exposure of sprayed pesticides as this action is the early step to prevent the sprayed pesticides entering through the skin.\textsuperscript{22} Result like variable self-pro-

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**Table 5. Final Model of Multivariate Analysis (Multiple Logistic Regression Analysis)**

| Variable               | B   | p value | OR    | CI 95% Lower | CI 95% Upper |
|------------------------|-----|---------|-------|--------------|--------------|
| Pesticide types        | 0.961 | 0.089  | 2.614 | 0.863        | 7.915        |
| Working time           | 2.644 | 0.000  | 14.072 | 4.113        | 48.149       |
| Use of gloves          | 0.776 | 0.199  | 2.173 | 0.665        | 7.101        |
| Hand washing behavior  | 1.469 | 0.053  | 4.346 | 0.979        | 19.305       |
| Constant               | -8.353 | 0.000  | 0.000 |              |              |
tector use was previously found on variable self-protector use in form of long sleeve shirt. Bivariate analysis in this study showed no significant relation between the use of long sleeve shirt and cholinesterase enzyme activity. Result of this study was aligned with the prior study which did not obtain significant relation between the use of long sleeve shirt and cholinesterase enzyme activity in farmer’s blood.\(^{23}\)

While applying pesticides, farmers should always use gloves as self-protector. The previous study had explained that disobedience to the use of self-protector would increase risk of decrease of cholinesterase activity in blood.\(^{21,24}\) The result of bivariate analysis showed any significant relation between the use of gloves and cholinesterase enzyme activity of fertile women farmers based on OR score, meanwhile multivariate analysis showed that variable the use of gloves was the factor contributing to cholinesterase enzyme activity. Most fertile women farmers had used gloves while working at rice field, but all the gloves used by the farmers were made of fabric. Gloves made of fabric are easily traversed by liquid into the skin, so farmers should replace it by gloves made of rubber material which is liquid proof.

Washing hands before eating is self-protection in form of personal hygiene behavior to avoid exposure of any substance to enter through mouth.\(^{20,25}\) Hands unwashed before eating will bring pesticides attached on hands, so this can cause pesticides contaminate food and enter through alimentary canal. Time needed for pesticides to enter into the body through oral may happen fast from 30 to 90 minutes.\(^{11}\) Therefore, fertile women farmers should always keep their hands clean before eating whether at rice field or not. Bivariate analysis showed any significant relation between hand-washing behavior of farmers and cholinesterase enzyme activity, meanwhile multivariate analysis showed that hand-washing activity was the factor contributing to cholinesterase enzyme activity of fertile women farmers. Such results were aligned with the prior study that found significant relation between hand-washing behavior and cholinesterase enzyme activity in blood.\(^{26}\)

Similar to the previous variable, variable bathing behavior was one of behaviors that belong to personal hygiene. However, bivariate analysis showed no any significant relation between farmer’s bathing behavior and cholinesterase enzyme activity in blood of fertile women farmers. Based on this study, most fertile women farmers had good behavior by taking a bath after going home from farming. This was an act of fertile women farmers to remove any pesticides attached on the body. This was aligned with the prior results of study that did not found significant relation between personal hygiene and cholinesterase enzyme activity in blood.\(^{15}\) Bathing is one of acts to remove pesticide exposure attached on the body, therefore good practice of bathing can be taken after using pesticides, such as spraying, mixing or doing other farming activities that contain activities using pesticides.\(^{18}\)

**Conclusion**

Based on analysis results, there are four variables related to the decrease of cholinesterase enzyme activity of fertile women farmers at Kedunguter Village namely pesticide types, working time, glove using and hand-washing behaviors. Variable working time is the most dominant variable to the decrease of cholinesterase activity ≤ 87.5\%.

**Recommendation**

Working time is the dominant variable influencing cholinesterase enzyme activity in blood of fertile women farmers, so health agency and agriculture agency should provide counseling for the farmers to not spend time of farming more than six hours per day. The working time for six hours per day among fertile women farmers can decrease their work productivity and income. An act to maintain and increase productivity of fertile women farmers can be performed by any multisectoral activities between health agency, agriculture agency and other social institutions in Brebes District. Activities that can be held are entrepreneurship training or opening job opportunities that can maintain productivity as well as increase income of fertile women farmers at Kedunguter Village.

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