

Complete blood count, cholinesterase, and renal function of organophosphate sprayers in Kulonprogo

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<p>Article history</p> <p>Received: Nov 14, 2016 Received in revised form: May 11, 2017 Accepted: May 12, 2017 Available online: August 20, 2017</p> <p>Corresponding author</p> <p>Idha Arfianti Wiraagni Forensic and Medicolegal Departement, Faculty of Medicine, UGM, Farmako Sekip Utara, Yogyakarta 55281</p> <p>Phone: 081328067816 Email: arfianti.idha@gmail.com</p>	<p>Abstract</p> <p>Background: There are several risks to health associated with pesticide use. The use of Personal Protective Equipment (PPE) can minimize pesticide exposure to farmers. The aim of this study was to determine the basic characteristics of farmers, blood parameters (complete blood count, cholinesterase, and creatinine), patterns of pesticide use, and the use of PPE.</p> <p>Methods: This research was a cross-sectional study, with total sampling method. The data were taken from all farmers in Kulonprogo, Yogyakarta, Indonesia. Case group was organophosphate sprayers that have sprayed organophosphate for more than 1 year.</p> <p>Results: In case of group, there were 36 farmers (31 male and 5 female), while in control group, there were 11 persons (4 male and 7 female). The mean blood cholinesterase level in the exposed group was 7.8 ± 2.01 Ku/L and in the control group 8.7 ± 1.56 Ku/L. The mean of exposed group blood urea nitrogen: 12.08 ± 3.88 mmol/L and control Group: 11.4 ± 3.11 mmol/L. The mean of blood creatinine was within normal limits (case group: 0.9 ± 0.17 mg/dl and control group: 0.7 ± 0.19 mg/dl), but there were significant differences between them ($p : 0.015$).</p> <p>Conclusions: The results of a complete blood count, cholinesterase, and renal function in the organophosphate sprayers In Kulonprogo were within normal limits. There was an increase of creatinine levels on exposed group significantly, although still within normal limit. They have sufficient rest period for farmers (1 month) in every planting season. It is necessary to educate farmers about the importance of using PPE and management of acute pesticide poisoning.</p>
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Keywords: complete blood count, cholinesterase, renal function, organophosphate sprayer, creatinine
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Introduction

Organophosphate use was very high compared to organochlorines, as insecticides, fungicides, herbicides, and additives in the industry (1) (2). Around the world, approximately three million people are exposed to organophosphate each year, with up to 300.000 fatalities among others. The World Health Organization (WHO) estimates there are about 25 million cases of pesticide

poisoning each year, or about 68.493 cases every day. The toxicity comes from the exposure during work, either unintentional or intentional, including murder and suicide (3) (4). The use of organophosphate has caused serious health problems in the last three decades in developing countries. However, the real scope of the problem was very difficult to detect because of various reasons. Farmers with mild organophosphate

poisoning were not reported to health officials, because of long distances, high cost, or fear of losing their job. Poisoning of health workers in rural areas is often difficult to diagnose, because the symptoms are common, such as dizziness, nausea, vomiting (5).

Based on the United Nations Press Release in 2004, about 1 to 5 million cases of poisoning are estimated across the world each year, and several thousand of these cases occur among farmers. An estimated 3% of the farmers had an episode of acute exposure to organophosphates. The majority of poisoning cases occurred in developing countries, due to lack of hygiene, information, personal protective equipment, and limitations of adequate control of the government. Developing countries use only 25% of the pesticides used all over the world, but the incidence of death related to organophosphate use is as high as 99% (5). Some studies suggest the existence of similar problems in Africa (6) (7), Asia (8) (9) (10) (11), Europe(12) (13) (14) , and American (15) (16) (17) (18).

Many studies reported that farmers often suffer poisoning, due to routine exposure of organophosphate that enters through inhalation or dermal contact. Organophosphate poisoning can be acute or chronic. The severity of toxicity depends on the type of pesticide, pesticide dose, duration, and the frequency of exposure. Its intensity is influenced by the size of the rice fields, application mode, climatic condition, spraying skills, and use of personal protective equipment (19).¹⁹ Among pesticides, organophosphate and carbamate cause mild to severe toxicity. The main mechanism of action is to inhibit acetylcholinesterase (AChE) (20) (21). Due to AChE inhibition, levels of acetylcholinesterase fall, thereby raising acetylcholine levels in the synaptic gap. This can cause nicotinic, muscarinic, and intoxication symptoms of peripheral than central nervous system (22). As well as the organophosphates, carbamates also inhibit the cholinesterase enzyme and cause similar clinical symptoms, but the duration of toxicity is shorter (23).

Clinical symptoms of acute intoxication include, increase in secretion, bronchoconstriction, myosis, diarrhea, bradycardia, muscular fasciculations, CNS depression, convulsions, cyanosis, and coma (20). Acute effects appear soon in the 24 hours after exposure. In general, acute poisoning is reversible if treated immediately with appropriate management. However, it would be fatal if the

therapy is not given (23). A decrease in hemoglobin and an increase in white blood cells in the affected farmers exposed by organophosphate was reported by Hundekari *et al.* (2013) (24). These conditions will expose patients to infection and increase the risk of anemia. Several studies have reported a significant association between exposure to organophosphate with abnormalities of the blood profile, cholinesterase levels, and the incidence of acute renal failure (25) (26).

Various blood parameters including blood cholinesterase levels were studied in organophosphate sprayers who were not previously investigated, especially in Kulonprogo, Yogyakarta, Indonesia. The general objective of this research was to know the description of complete blood profile, cholinesterase, and renal function of organophosphate sprayers in Kulonprogo. Application of the results of this study was to assess the work safety and health of organophosphate sprayers, also to educate the importance of using personal protective equipment to prevent acute poisoning, and the importance of continuous monitoring to determine insecticide accumulation in the body.

Materials and methods

This research was conducted with an observational design with cross-sectional approach. Blood samplings were performed on June 1, 2016, to examine complete blood profile, cholinesterase, and renal function. The sampling method in this study was total sampling, which captured all the organophosphate sprayers in Kulonprogo, Yogyakarta, Indonesia. After taking informed consent, a complete history was taken followed by physical examination and blood sampling of all of the subjects. Blood was sent to the laboratory to assess complete blood count, cholinesterase, and renal function. The exposed group was the organophosphate sprayers in Kulonprogo, who have sprayed organophosphates more than 1 year. The control group was non-farmers citizens of Kulonprogo. In this study, there were 36 people in the exposed group and 11 people in control group. Statistical analysis was univariate to describe the basic characteristics, history of pesticide spraying, PPE use history, and laboratory results. For the laboratory results, we analyzed differences between two groups with independent sample t-test.

Results

1. Basic Characteristics of Research Subjects

In Kulonprogo, we obtained 31 men (86.1%) and 5 women (13.8%) in the exposed group. While in the control group, there were 4 men (36.4%) and

7 women (63.6%). The sex, age, and education level distribution of the exposed group and control group are as per Tables 1 and 2 respectively.

Table 1: Basic characteristics of exposed group

Variable	N (%)
Sex	
-Female	5(13.8)
-Male	31(86.1)
Age	
<18 years	0(0)
-18-21 years	0(0)
-22-40 years	9(25)
-41-60 years	20(55.5)
>60 years	7(19.4)
Education	
-Elementary	18 (50)
-Junior high school	8(22.2)
-Senior high school	10(27.8)
-Bachelor/ vocational	0(0)

Table 2: Basic characteristics of control group

Variable	N (%)
Sex	
-Female	7 (63.6)
-Male	4 (36.4)
Age	
<18 years	0 (0)
-18-21 years	1 (9.1)
-22-40 years	0 (0)
-41-60 years	9 (81.8)
>60 years	1 (9.1)
Education	
-Elementary school	3 (27.3)
-Junior high school	3 (27.3)
-Senior high school	2 (18.2)
-Bachelor/ vocational	3 (27.3)

2. Physical Examination Results of Research Subjects

Physical examination assessed in this study covered the body mass index, blood pressure, and the presence or absence of tremor. The results of the examination of body mass index in the exposed group, 3 people (8.3%) were underweight, 30 people (83.3%) were normal, 1 (2.8%) was overweight, and 2 (5.6%) were obese. In the control group, 4 (36.4%) were underweight, 4 (36.4%) were normal, 2 (18.2%) were overweight, and 1 (9%) was obese. From the exposed group, 22 people (61.1%) showed normal blood pressure, and the remaining 14 (38.9%) showed high blood pressure. Meanwhile, in the control group, the normal result was obtained in 8 (73.7%) and 3 were

(27.3%) high. On the tremor examination, all of the farmers had tremors. While the control group, there were positive results in 8 (73.7%) people and negative results in 3 (27.3%) people (Table 3 and Table 4). Eleven farmers had experienced symptoms of acute poisoning, like dizziness, and nausea.

Table 3: Physical examination of exposed group

Variable	N (%)
BMI	
- Underweight (<18.5)	3 (8.3)
- Normal (18.5 – <25)	30 (83.3)
- Overweight (25 - <30)	1 (2.8)
- Obese (≥30)	2 (5.6)
Blood Pressure	
- Low (≤90/60)	0 (0)
- Normal	22 (61.1)
- High (≥140/90)	14 (38.9)
Tremor	
- Positive	36 (100)
- Negative	0 (0)

Table 4: Physical examination of control group

Variable	N (%)
BMI	
- Underweight (<18.5)	4 (36.4)
- Normal (18.5 – <25)	4 (36.4)
- Overweight (25 - <30)	2 (18.2)
- Obese (≥30)	1 (9)
Blood pressure	
- Low (≤90/60)	0 (0)
- Normal	8 (73.7)
- High (≥140/90)	3 (27.3)
Tremor	
- Positive	8 (73.7)
- Negative	3 (27.3)

3. Laboratory Result of Research Subjects

Laboratory tests assessed in this research, covered hemoglobin level, erythrocyte number, leukocyte number, platelet number, blood urea nitrogen level, creatinine, and cholinesterase levels. Hemoglobin level of the exposed group found to be low in 3 (8.3%) and normal in 33 (91.7%). In the control group, 10 (90.9%) was found normal and 1 (9.1%) was found high. Erythrocyte level in the exposed group was low in five people (13.9%), normal in 30 (83.3%), and high in 1 (2.8%). While in the control group 10 (90.9%) was found normal, and 1 (9.1%) was found high (Table 5 and Table 6).

Table 5: Laboratory result of exposed group

Variable	N (%)	Variable	N (%)
Hemoglobin level		Blood Urea Nitrogen Level	
-Low	3 (8.3)	-Low	0 (0)
-Normal	33 (91.7)	-Normal	34 (94.4)
-High	0 (0)	-High	2 (5.6)
Erythrocyte number		Creatinine level	
-Low	5 (13.9)	-Low	0 (0)
-Normal	30 (83.3)	-Normal	35 (97.2)
-High	1 (2.8)	-High	1 (2.8)
Leukocyte number		Cholinesterase level	
-Low	0 (0)	-Low	
-Normal	35 (97.2)	-Normal	1 (2.8)
-High	1 (2.8)	-High	35 (97.2)
Thrombocyte number			0 (0)
-Low	0 (0)		
-Normal	36 (100)		
-High	0 (0)		

Table 6: Laboratory result of control group

Variable	N (%)	Variable	N (%)
Hemoglobin level		Blood urea nitrogen level	
-Low	0 (0)	-Low	0 (0)
-Normal	10 (90.9)	-Normal	11 (100)
-High	1 (9.1)	-High	0 (0)
Erythrocyte number		Creatinine level	
-Low	0 (0)	-Low	0 (0)
-Normal	10 (90.9)	-Normal	11 (100)
-High	1 (9.1)	-High	0 (0)
Leukocyte number		Cholinesterase level	
-Low	0 (0)	-Low	0 (0)
-Normal	10 (90.9)	-Normal	11 (100)
-High	1 (9.1)	-High	0 (0)
Thrombocyte number			
-Low	0 (0)		
-Normal	11 (100)		
-High	0 (0)		

Leukocyte number in exposed group was normal with 35 people (97.2%) and high in 1 (2.8%). In the control group, 10 people (90.9%) were normal and high in 1 (9.1%). Platelet numbers were found to be normal in all subjects in both groups. The level of blood urea nitrogen in exposed group was found to be normal in 34 (94.4%) and high in 2 (5.6%). While in the control group, it was found normal in all subjects. Creatinine level in farmer group was found to be normal in 35 (97.2%) and high in 1 (2.8%). While the control group, it was found normal in all subjects. Cholinesterase

level in farmer group was found to be low in 1 (2.8%) and normal in 35 others (97.2%). In the control group, normal values were obtained in all subjects (Table 5 and Table 6).

In this study, to know more clearly the average differences between the two groups, we conducted independent test sample t-test (Table 7). From all results, there were significant differences in creatinine level. The exposed group has higher creatinine level (0.91 ± 0.17), compared with the control group (0.77 ± 0.11) (Table 7).

Table 7: Mean differences between exposed and control group

Variable	Mean of Exposed Group	Mean of Control Group	Independent Sample T-Test
Hemoglobin level	14.36±1.05	14.21±0.9	0.69
Eritrocyte number	4.89±0.42	4.95±0.43	0.74
Leukocyte number	7.69±1.58	8.69±1.88	0.08
Trombocyte number	2.5±0.43	2.7±0.69	0.29
Blood urea nitrogen level	12.08±3.88	11.4±3.11	0.6
Creatinine level	0.91±0.17	0.77±0.11	0.015
Cholinesterase level	7.79±2.01	8.74±1.56	0.12

Table 8: History of pesticides usage

Variable	N (%)	Variable	N (%)
Total spraying time		Duration of spraying	
-<5 years	1 (2.8)	- < 1 hour	17 (47.2)
-5-10 years	27 (75)	- 1-2 hours	14 (38.9)
->10-20 years	5 (13.9)	- >2 hours	5 (13.9)
->20 years	3 (8.3)	Spraying frequency	
Last time spraying		- everyday	6 (16.7)
-< 1 week	32 (88.9)	- once in two days	22 (61.1)
-1-2 weeks	4 (11.1)	- once in > 2 days	8 (22.2)
- 2-3 weeks	0 (0)	Types of pesticide used	
Spraying area		-Karbamat	25 (69.4)
- <500 m ²	7 (19.4)	-Organofosfat	23 (63.9)
- 500-1000 m ²	13 (36.1)	-Pirol	15 (41.7)
->1000-2000 m ²	13 (36.1)	-Azol	9 (25)
- >2000 m ²	3 (8.3)	-Piretroid	9 (25)

Table 9: History of personal protective equipment use

Variable	N (%)	Variable	N (%)
PPE type		PPE washing methode	
-Masker	23 (63.9)	-with detergent	28 (100)
-Gloves	5 (13.9)	-without detergent	0 (0)
-Boots	3 (8.3)	Reason of not using PPE	
-Glasses	0 (0)	-not practical	10 (27.8)
-Hat	28 (77.8)	-muddy	25 (69.4)
Frequency of PPE washing		-feeling healthy	2 (5.6)
-every time used	25 (83.3)	-can not breathe freely	1 (2.8)
-after being used more than one	3 (10)	-the area is not extensive	2 (5.6)
-none	2 (6.7)		

4. History of Pesticides Use

History of pesticide use of organophosphate sprayer was assessed by several variables, such as total spraying time, the last time spraying, the area being sprayed, the duration of spraying time, spraying frequency, and the type of pesticide used. One person (2.8%) has been spraying for less than five years, 27 people (75%) for 5 to 10 years, 5 people

(13.9%) for over 10 to 20 years, and the remaining 3 people (8.3%) had sprayed more than 20 years. The land area being sprayed was <500 m² in 7 people (19.4%), 500 to 1000 m² in 13 people (36.1%), 1000 up to 2000 m² of 13 people (36.1%), and the rest was more than 2000 m² (8.3%). The majority of farmers (88.9%) sprayed less than one week ago. While the rest (11.1%) were about 1 to 2 weeks ago.

Spraying time was less than 1 hour in 17 (47%), 1 to 2 hours in 14 (38.9) and more than 2 hours in the rest (13.9%). Most farmers sprayed their

Types of pesticides used were quite a lot and most of the farmers use the same pesticides. Organophosphate was used by all farmers (100%), followed by carbamate, pyrrole, azole, and pyrethroid by 25 (69.4%), 15 (41.7%), 9 (25%), and 9 (25%) farmers respectively. Other pesticides were also used, such as B. Bassiana (organic pesticides), antranilamide, ivermectin, and nereistoxin. They have sufficient rest period for farmers (1 month) in every planting season (Table 8).

5. History of Personal Protective Equipment Usage

The history of personal protective equipment (PPE) use on exposed use was judged from the type of PPE, the frequency of PPE washing, PPE washing methods, and reasons for not wearing

fields once every 2 days (61.1%), some of them spray every day (16.7%) and others were once in more than 2 days (22.2%) (Table 8).

PPE. From 36 subjects, 30 were at least one PPE. Twenty-three people (63.9%) wore a mask, 5 (13.9%) wore gloves, 3 (8.3%) wore boots, and 28 people (77.8%) wore a hat. Of the 30 people who use PPE, 25 people (83.3%) washed the PPE right after each use, 3 (10%) farmers washed PPE after the PPEs were worn more than once, and 2 (6.7%) did not wash PPE. Overall PPE washing was done by detergents. As for the reasons for not wearing PPE, 10 (27.8%) admitted that PPE was not practical, 25 people (69.4%) justified the ground was muddy (It was not safe to wear boots), thirdly 2 (5.6%) of them felt healthy without the use of PPE, 1 (2.8%) was not able to breathe freely, and 2 (5.6%) of them said because of sprayed land was not extensive (Table 9).

Discussion

The majority of the exposed group were men, as working in agriculture requires high energy. Women's role was complimentary such as small tasks, like watering or spraying the plants. This is consistent with other researchers in Indonesia which had 229 farmer respondents with one woman among them.²⁷ Most of them were in the age range of 41-60 years, it is also consistent with the result in the previous study with 229 farmers, the mean age was 38.4 ± 11.4 years (27). Another characteristic that almost all farmers had lower education level. The low level of education and low socioeconomic status, resulting in a lack of knowledge, especially in the prevention of organophosphate poisoning. In a village located in the rural area of Nicaragua, farmers have lower cholinesterase levels compared to other professions, because of the lack of knowledge to protect themselves from organophosphate exposure (28).

The majority of farmers in this study had normal body mass index. This is consistent with a variety of physical activity done by the farmer, thus burning fat that can lead to obesity. Furthermore, the inspection of the chest, abdomen, and blood pressure was also within normal limits, although there were small farmers who suffered from hypertension associated with aging which has been advanced. In the absence of an adequate barrier to acetylcholine in the synaptic cleft, this

can lead to the continuous passage of the impulse, then manifest as tremor. This is an effect of organophosphate on the nervous system, which is one of the negative impacts on the body. All farmers in the study showed tremor symptoms. In addition, tremor is a mild sign of chronic organophosphate poisoning (29).

Based on the results above, we can see that the average of all laboratory results in both groups was within normal limits, but the mean of creatinine level in exposed group was higher significantly compared to control group. The results of complete blood count were within normal limit. Creatinine is a waste product of creatine phosphate breakdown that occurs in muscles. This substance could rise if there is damage to kidney cells. Creatinine is always together with urea, so a ratio/comparison was created. This ratio can thoroughly assess renal function. Based on the ratio of blood urea nitrogen/creatinine obtained normal results. Therefore, we can conclude that there were no significant body physiological abnormalities in both groups, although there was a rise in creatinine of exposed groups. These results were consistent with cholinesterase level among farmers, there was only one farmer that had low cholinesterase level. This means that there were no significant organophosphate residues in the body of farmers that can cause abnormalities of blood parameters. It was also possible there was a decrease in cholinesterase, but there was a sufficient rest period between the time of

spraying, resulting in new cholinesterase synthesis. Research in the North, Midzone Kan Yunis, Rafah and Gaza Strip also showed the differences in creatinine levels between two groups and exposed group had a higher value (30).

In previous studies of parameters such as leukocyte number, hematocrit number, and platelet number, there was a significant increase in the group of farmers who do not wear Personal Protection Equipment (PPE) completely. When the decrease in cholinesterase level was measured, there was a significant reduction in the exposed group. The study was consistent with the results of this study, in which the average of cholinesterase was normal, so the outcome of complete blood count was also under normal circumstances (30).⁰

Based on the type of pesticide used, all of the farmers use organophosphates. Organophosphate pesticides are combined with other types. The combination of pesticides is done, because of the emergence of plant pests that vary from time to time. The results were consistent with Kishi et al., (1995) with the subject of farmers in Indonesia, that some of the pesticides that are used include, organophosphates, carbamates, and pyrethroids. The class of organophosphates used were class IB and II according to the WHO classification. Group IB is a dangerous group and class II is a group that is quite dangerous (27).

None of the farmers wore complete personal protective equipment. Personal protective equipment that is frequently and easily used in the field was a hat. They had some reasons for not wearing full personal protective equipment. The main reason was the fear of slip or fall because the soil was muddy. According to previous studies, farmers in Indonesia did not wear complete personal protective equipment. There are situations where they were potentially exposed such as, when they were diluting pesticides with water, spraying on crops (especially if the direction of spraying against the wind), exposing body with the pesticide disposal waste water, and harvesting crops (27).

Conclusion

The results of a complete blood count, cholinesterase, and renal function in the organophosphate sprayers in Kulonprogo were within normal limits. There was an increase of creatinine levels on exposed group significantly, although still within normal limit. There was the tendency of creatinine rising in the exposed group, which was statistically significant. Some advice that

can be given is to conduct a new research to examine blood parameters before and after spraying, so if there is a decrease in cholinesterase levels, it can be detected quickly. It should have the control group be at least twice the size of the exposed group, to generate a strong causal relationship. Keep checking the content levels of organophosphates in the onion and soil, as well as cholinesterase levels in the body of onion consumers.

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