ASSESSMENT OF HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT NAKHON NAYOK PROVINCE, THAILAND

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ABSTRACT: This study aims to assess health effects caused by organophosphate pesticides exposure among farmers (n=35) and non-farmers (n=35) in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok province. The research design of this study was a cross-sectional study during pesticide application period from January to March 2013. Questionnaire and Test-mate ChE (Model 400) for blood cholinesterase levels of both blood enzymes erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE) were used as measurement tools. Participants were consisted of 25 male and 45 female; average age (±SD) was 42.63 (±10.41) years old. The results showed that AChE levels of farmers was likely lower than non-farmers and PChE levels in the farmer group was significantly lower than those in non-farmer group (t-test, $p<0.001$). The association between AChE levels and PChE levels were likely low negative correlation. Years of using pesticides were significantly associated with PChE levels (Chi- square, $p<0.05$). The farmers were significantly associated with increase eye symptoms, central nervous system (CNS) symptoms, respiratory system symptoms, and gland symptoms (Chi- square, $p<0.05$). The AChE level was significantly associated with CNS symptoms (Chi- square, $p<0.05$) . The PChE level was significantly associated with eye symptoms, CNS symptoms, respiratory system symptoms, and gland symptoms (Chi-square, $p<0.05$). In conclusion, farmers may be getting higher risk than non-farmers living nearby/around farming area. An appropriated self-prevention from pesticides exposure should be recommended to farmers and non-farmers; particularly, a proper use of personal protective equipment (PPE) should be introduced to farmers.

Keywords: Organophosphate, Cholinesterase activity, Health effects, Thailand

INTRODUCTION

Thailand is an agricultural country that produces one of the largest quantities of rice in the world. In 2009, the total area of rice farming in Thailand covered approximately 26 million acres across the country [1]. Organophosphate (OP) pesticides contain chemicals which are primarily used in agriculture [2]. Besides being rapidly decomposed, these components are somewhat non persistent in the environment. However, they are described as being highly and acutely toxic [3]. As a result, farmers, as well as the general public, may run the risk of exposure to such substances which can be exposed to in several ways such as breath, oral cavity, and skin [4].

OP pesticides include a high toxicity on humans due to the fact that they act as acetyl cholinesterase inhibitor, resulting in the blockage of the nervous system [5]. The activity of cholinesterase enzymes in the blood can be measured used as a biomarker for the effect of organophosphates. AChE is characteristically used as an indicator for chronic exposure. On the contrary, PChE turnover is much more rapid. PChE is a more efficient short-term indicator [6]. A lot of methods can be used to test cholinesterase level. However, normal method used was a screening test, which should be confirmed by

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Ellman methods [7]. Therefore, this research aims to find the association between health effects and organophosphate pesticides exposure among rice farmers and non-farmers by using blood cholinesterase levels; both blood enzymes erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE). Cholinesterase level was tested by Ellman method; Test-mate ChE (Model 400).

MATHERIALS AND METHODS

The research design of this study is a cross-sectional study. Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok province, Thailand, was purposively selected because it is occupied by the largest number of agriculturists as well as contains the most rice paddies in the District [8]. Based on sample size calculated by using the formula for the sample size for the mean [9], target populations were divided into two groups; farmer group (n=35) and non-farmer group (n=35). Inclusion criteria for the farmer group were rice farmers, both male and female, aged 18 to 59. They were all growing rice, applying organophosphate (OP) pesticides in paddy areas, as well as loading, mixing, or/and spraying a day (24 hours) before blood collection. Inclusion criteria for the non-farmer group were non-rice farmers, both male and female, aged 18 to 59. They were all non-daily farm workers, had no contact with pesticide application within three months both from their household or planting. Those with a history of liver failure, cardiovascular disease, taking anti-malarial drugs, malnutrition, and taking amphetamine, were excluded. The representatives were recruited as subjects from each house (one subject per household). The simple random sampling was done by drawing in accordance with the criteria is used to get target number of sample (70 subjects).

Measurement tools

1) Questionnaire

The principal researcher assessed the subjects by site visits and observations. Questionnaire is separated into four parts; part 1: obtain general information and individual background; part 2: obtain pesticides use; part 3: obtain related exposure factor, and part 4: obtain health information.

2) Blood test by Test-mate ChE (Model 400), EQM

The Test-mate ChE Cholinesterase Test System is on the basis of Ellman method. Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or PChE, producing carboxylic acid and thiocholine, respectively, with reaction to the Ellman reagent (DTNB, dithionitrobenzoic acid) so as to create a yellow color that is gauged spectrophotometrically at 450 nm. The rate of color formation is in proportion to the amount of either AChE or PChE [10]. Nurses collected 20μL of blood per person from both farmers and non-farmers in an air-conditioned room, at the primary health care center, in which optimum temperature was controlled at less than 30 C as recommended by Test-mate ChE Cholinesterase Test System (Model 400) specification. For the farmers, their blood was collected after 24 hours of the last of pesticide application (loading, mixing, and/ or spraying) [11]. And Non-farmer's blood was collected during their free days. The analysis of cholinesterase level in erythrocyte and plasma using Test-mate was conducted by the researcher.

Data analysis

Interpret level of cholinesterase: if it values less than or equal 50% from normal it indicates possible pesticide poisoning and should be removed from the exposure and/or treated with anticholinergics [12]. A cholinesterase level of more than 50% from the normal value is considered normal. Using the licensed SPSS version 17 for windows, general characteristics and socio-demographic were described by frequency, percentage, and mean. Independent T-Test was used to determine the difference between blood cholinesterase levels of farmer group and non-farmer group. Chi-square was used to find relation between pesticides use and blood cholinesterase level and a relationship between health effects and level of blood cholinesterase. An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. OR equal 1 indicated that exposure does not affect odds of outcome. OR more than 1 that means exposure associated with higher odds of outcome. OR less than 1 indicated that exposure associated with lower odds of outcome. The OR was used to explain a relationship between health effect and pesticide exposure.

Ethic consideration

The experimental protocol was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Sciences Group, Chulalongkorn University with the certified code no.002/2013.

Table 1 Subjective symptoms related to organophosphate pesticides exposure

Symptoms	Farmers (n=35) n(%)	Non-Farmers (n=35) n(%)	OR (95% CI)
Skin symptoms			
Skin rash/ itching/ burning	17 (48.6%)	10 (28.6%)	2.361 (0.879-6.345)
Tingling/Numbness of hands	12 (34.3%)	12 (34.3%)	1.000 (0.373-2.683)
Muscular twitching and cramps	11 (31.4%)	9 (25.7%)	1.324 (0.467-3.750)
Eye symptoms			
Blurred vision*	22 (62.9%)	12 (34.3%)	3.244 (1.219-8.629)
Lacrimation*	16 (45.7%)	3 (8.6%)	8.982 (2.311-34.910)
Irritation*	24 (68.6%)	14 (40%)	3.273 (1.224-8.748)
Central nervous system			
Headache*	29 (82.9%)	16 (45.7%)	5.740 (1.906-17.282)
Dizziness*	28 (80.0%)	10 (28.6%)	10.000 (3.308-30.230)
Drowsiness	13 (37.1%)	9 (25.7%)	1.707 (0.614-4.744)
Slurred speech	0 (0%)	2 (5.7%)	NC
Ataxia	1 (2.9%)	0 (0%)	NC
Trembling of hands	3 (8.6%)	2 (5.7%)	1.547 (0.242-9.878)
Irritability*	12 (34.3%)	4 (11.4%)	4.043 (1.154-14.164)
Memory problem	1 (2.9%)	4 (11.4%)	0.228 (0.024-2.151)
Respiratory system			
Wheezing*	13 (37.1%)	5 (14.3%)	3.545 (1.102-11.411)
Dyaspnea	10 (28.6%)	5 (14.3%)	2.400 (0.725-7.949)
Bronchorrhea	1 (2.9%)	0 (0%)	NC
Running nose	0 (0%)	1 (2.9%)	NC
Shortness of breath	15 (42.9%)	10 (28.6%)	1.875 (0.695-5.061)
Gastrointestinal system			
Anorexia	6 (17.1%)	4 (11.4%)	1.603 (0.410-6.264)
Vomiting	4 (11.4%)	2 (5.7%)	2.129 (0.364-2.459)
Abdominal cramps	8 (22.9%)	5 (14.3%)	1.778 (0.518-6.097)
Fecal incontinence	1 (2.9%)	0 (0%)	NC
Urinary system			
Loss of urinary control	1 (2.9%)	0 (0%)	NC
Glands			
Hyper salivation	4 (11.4%)	0 (0%)	NC
Sweating*	14 (40%)	2 (5.7%)	11.000 (2.267-53.372)

*Significant at 0.05 probability level
NC- not calculated

RESULTS

General characteristics and socio-demographic

35 farmers who were participated in this study were male (40%) and female (60%). The average age (±SD) was 42.40 (±9.42). Most of them finished primary school (62.9%). Sixty percent of them had an income of about 30,000 - 60,000 THB (1 USD ~ 30 THB) per year. More than half of the participants (60%) were not smokers but they were drinkers (62.9%).

In the non-farmer group, there were both men (31.4%) and women (68.6%). The average age (±SD) was 42.86 (±11.44) years old. Half of them graduated from primary school (51.4%). About sixty six percent of them had an income of about 30,000 – 60,000 THB annually. Up to 85.7% of them were non-smokers and 68.6% were non-drinkers, which were a higher smoking rate compared to those of the farmers.

Pesticides use

The most common pesticides used were organophosphates and carbamates such as chlorpyrifos, profenofos, metamidofos, fenobucarb, carbosulfan etc. The farmers who participated in the survey said that they mixed more than one kind of hazardous pesticides. The average (±SD) year of using pesticides was 18.64 (±11.58). The average working hours were about 5.1 hours per day and the average of cultivation area was 30.74 (±19.81) rais (1 rai = 0.4 acre). 37% of the farmers used the

Table 2 Association between ChE levels (AChE and PChE) and reported health symptoms in participants (n=70)

Health symptoms	AChE (%N)		PChE (%N)	
	χ^2	*P-value*	χ^2	*P-value*
Skin symptoms				
Skin rash/ itching/ burning	1.143	0.285	2.280	0.131
Tingling/Numbness of hands	0.794	0.373	0.320	0.572
Muscular twitching and cramps	0.810	0.366	0.055	0.814
Eye symptoms				
Blurred vision	0.490	0.484	2.784	0.095
Lacrimation	0.177	0.752	10.928	0.001*
Irritation	0.154	0.695	8.541	0.003*
Central nervous system				
Headache	2.600	0.107	4.895	0.027*
Dizziness	6.077	0.014*	11.690	0.001*
Drowsiness	0.000	0.986	0.195	0.659
Slurred speech	0.610	1.000	1.217	0.526
Ataxia	3.424	0.229	1.717	0.371
Trembling of hands	0.025	1.000	1.205	0.353
Irritability	0.829	0.498	8.875	0.003*
Memory problem	1.595	0.582	3.182	0.074
Respiratory system				
Wheezing	0.333	0.536	5.962	0.015*
Dyaspnea	0.157	0.734	4.272	0.039*
Bronchorrhea	0.301	1.000	1.717	0.371
Running nose	0.301	1.000	0.599	1.000
Shortness of breath	0.583	0.445	5.923	0.015*
Gastrointestinal system				
Anorexia	1.944	0.221	0.041	1.000
Abdominal cramps	0.567	0.476	0.555	0.531
Fecal incontinence	3.424	0.229	1.717	0.371
Urinary system				
Loss of urinary control	3.424	0.229	1.717	0.371
Glands				
Hyper salivation	0.011	1.000	7.179	0.016*
Sweating	0.198	0.748	12.732	0.000*

*Significant at 0.05 probability level

pesticides by mixing, loading, and spraying. They mostly sprayed pesticides using by gasoline engine power sprayer pumps connected to pesticide tanks (60%) and hand sprayers (40%). Most farmers applied pesticides on their crops in the early morning and in late evening (65.7%).

About 91.4 % of the farmers read the label on the pesticide products and up to 82.9% of them used the proper amount of pesticides as stated on the label. While working in the fields, 88.6% of them did not eat and drink near the area and after using pesticides; about 97.1% of them washed their hands before eating. About 94% of them instantly took shower after finishing their farm work. They mostly washed their pesticide stained clothes and normal clothes separately (94.3%). When the products were used up, only 8.6% of the participants buried them while the rest of them (91.4%) did not and threw or burned them around the farming area.

Investigating the use of personal protective equipment (PPE) while applying pesticides in the paddy fields, every farmer indicated that they wore long sleeved shirts and long legged pants. Up to 97.1% of the participants wore hats and masks, only one participant had never worn a hat or a mask. Some farmers reported that they used gloves (37.1%). At the same time, only 5.7% and 8.6% of them wore boots and goggles respectively.

Health effects related to OP pesticides exposure

Table 1 indicates that farmers who were directly exposed to pesticides were inclined to have a significant increase in eye symptoms: blurred vision (OR=3.244, 95%CI 1.219-8.629), lacrimation (OR=8.982, 95%CI 2.311-34.910), and irritation (OR=3.273, 95%CI 1.224-8.748). The positive associations of farmers are significantly related to

central nervous system symptoms: headache (OR=5.740, 95%CI 1.906-17.282), dizziness (OR=10.000, 95%CI 3.308-30.230), and irritability (OR=4.043, 95%CI 1.154-14.164). Moreover, the study showed that farmers were significantly associated with an increase in wheezing (OR=3.545, 95%CI 1.102-11.411) and sweating (OR=11.000, 95%CI 2.267-53.372). The farmer group did not associate with skin symptoms, gastrointestinal system symptoms and urinary system symptom.

Prevalence of abnormal ChE levels both AChE and PChE

In farmer group, the prevalence of abnormal AChE levels was 31.4%. In non-farmer group, the prevalence of abnormal AChE levels was 14.3%. The results showed that the prevalence of abnormal AChE levels in the farmer group was likely more than in the non-farmer group.

According to PChE levels, 25.7 % of the farmers had normal PChE levels and 74.3% of them had abnormal PChE levels, while all of the non-farmers had normal PChE levels. The prevalence of normal PChE levels in the non-farmer group was higher than in the farmer group (t-test, *p=0.000*). The results showed the farmers may be getting more risk than the non-farmers.

Association between ChE levels and pesticide use

The study found that years of using pesticides were significantly associated with PChE levels (*chi-square, p* = 0.01). However, ChE activity was not associated with other agricultural work (hours of working per day, type of pesticides application, spraying method, and spraying time) and farming characteristics, and size of cultivating areas. Practicing of pesticide use and personal protective factors were not associated with ChE activity. Using PPE in the wrong practice and the improper use were not associated with ChE levels.

Association between ChE levels and health effects

Table 2 shows that the AChE level was significantly associated with dizziness (p=0.014) in central nervous system. But, the study was also showed that found the AChE levels were not significantly associated with skin symptoms, eye symptoms, respiratory system symptoms, gastrointestinal system symptoms, urinary system symptoms, and glands symptoms.

The study showed that PChE level was not associated with skin symptoms, gastrointestinal system, and urinary system. While The PChE level showed a significant association with lacrimation and irritation (p=0.001 and p=*0.*003, respectively). For central nervous system, the study revealed a significant association between PChE level and headache, dizziness, and irritability (p=0.027, p=0.001, and p=0.003, respectively). For respiratory system, the PChE level was significantly associated with wheezing, dyaspnea, and shortness of breath (p=0.015, p=0.039, and p=0.015 respectively). The association between PChE level and hyper salivation was found (p=0.016). Also there was an association between PChE level and the sweating (p=0.000).

DISCUSSION

From the statistically analysis presents the farmers were significantly associated with an increase eye symptoms. The results were similar to previous studies which showed that the predominance of eye symptoms found, 40% among pesticide sprayers, was significantly higher (p<0.01) as compared to the control group. The symptoms were found to be blurred vision, lacrimation, pain in eyes, red swollen eyes, and irritation of eyes [13]. Eyes are exposed to the external environment and thereby exposed to environmental contaminants. During agricultural operations, farm workers' eyes could be exposed to pesticides while spraying if there is a lacked of proper preventive steps. As a result, these chemicals are being absorbed through the eye tissue and enter the blood circulation. Exposure of unprotected eyes to pesticides results in the absorption in ocular tissue and potential ocular toxicity [14]. The present study found that the positive associations of farmers are significantly related to central nervous system symptoms. It is reasonable to show a health report regarding the symptoms caused by organophosphates poison to insects and mammals mainly by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The consequence is a loss of existing AChE which makes organs become over motivated by the incremental acetylcholine (ACh, the impulse-conveying substance) at the nerve ending. The enzyme is vital to regular control of the transmission of impulse from nerve fibers to smooth and skeletal muscle cells, glandular cells, as well as autonomic ganglia and within the central nervous system (CNS) [15]. Moreover, the study found that farmers were significantly associated with an increase in wheezing. In addition to specific cases, an inhalation hazard, that is to say intake into the lungs through nose or mouth during customary application of pesticides. When the pesticide formulation is volatile, therefore

respiratory protection would be necessary [16]. Moreover, the farmers were significantly associated with an increase of sweating which is one of the classic chronic signs [17].

In this study, it was found that the average AChE of farmers (2.63 ±0.55 U/ml) was lower than non-farmers (2.80 ±0.53 U/ml). The AChE activity in this study was higher than the AChE activity in the previous study which showed that AChE activity of farm workers was 1.36 ± 0.199 U/ml in males and 1.35 ± 0.19 U/ml in females. In the control group, AChE was 1.35±0.15 in males and 1.33 ±0.16 in females [18]. The possible reasons are either that different crops are associated with different AChE activity, or that the other studies were conducted in high risk areas. The study showed that farmers are likely to have lower AChE activity than non-farmers as stated in Simoniello's et al. [19] study that compared AChE between directly and indirectly exposed groups. Farmers are directly exposed to pesticides in a variety of ways. Pesticide products can be splashed or spilled on exposed skin during the pouring and mixing process of concentrated pesticide and during application, when spray or dust can contaminate the exposed skin or clothing. There is a risk of inhalation hazard since most pesticides are not sufficiently volatile, or due to the particle sizes generated during conventional application of sprays or dusts. The present study revealed that the prevalence of abnormal PChE levels in the farmer group was 74.3% but all of the non-farmers had normal PChE levels. The results are similar to other studies that mentioned that the PChE activity of farmer groups was lower than the control groups [20, 21].

The most common pesticides used in the study are similar with those in the study in the Rangsit agricultural area [22]. The study found PChE levels related to years of pesticide exposure. The result was consistent with previous study [23]. This finding may reflect an adaptive response to long-term challenges from OPs; in other words, chronic exposure to these compounds might lead to a higher enzyme activity (PChE induction) that would reduce OP binding to biological targets [24]. However, the study was not able to show that both AChE and PChE levels were associated with agricultural works, farming characteristics of pesticide use, and personal hygiene among farmers. Researcher's observations showed that the farmers used improperly PPE, improperly handing protective increasing the level of risk because the exposure level increasing the risk of exposure. Other studies support this, suggesting that wearing proper PPE is associated with a reduction in health hazards [25, 26]. Moreover, one of the previous studies found that the main risk factors related to agrochemical exposure depend on the use of pesticides. This includes inaccurate beliefs of from farmers regarding pesticide toxicity, the use of impaired spraying equipment, the lack of appropriate maintenance of spraying equipment, and the lack of protective gear or proper clothing [27]. There is a clear lack of uniform systems designed specifically for pesticide management in Thailand [28].

The results from the present study are similar to those of Midtling et al. [29] who found that many patients continued to report adverse health effects after erythrocyte cholinesterase had been removed from a previous affliction. The PChE level has found a significant association with eye symptoms, central nervous system symptoms, and respiratory system symptoms. With AChE no longer available to hydrolyze AChE, the neurotransmitter saturates the receptors and accumulates in the synapses, causing overstimulation and, later, blockage of further nerve impulses transmission. Thus, it is believed to be a good indicator of real neuronal activity. The rate of turnover for red blood cells is not quick (approximately 3 months). Therefore, AChE is used as an indicator of chronic exposure. For PChE, turnover is much quicker. PChE is a more effective short-term indicator because of its faster response to exposure. It is used as an indicator of recent, acute exposures [30].

CONCLUSION

The study showed that most of the farmers still use PPE in an improper way, or do not wear them at all during pesticide application. Reasons for this may include weather conditions such as heat and humidity may cause discomfort to the wearers since most protective apparel has low heat dissipation. Moreover, farmers cannot wear boots, which is an appropriate PPE while working in the field because they may damage the crops. Thus, the problem of wearing additional protective equipment in tropical countries is well known and has been commented upon over the years. The farmers in the study area may be getting more risk than non-farmers, while non-farmers had indirect exposure to pesticides. One way to reduce health effects from pesticide exposure is by wearing appropriate protection gears. Thus, there should be an intervention to reduce the risk by providing knowledge for the farmers to encourage practice of appropriate pesticides use and proper use of PPE while working with pesticides.

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