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FACTORS AFFECTING CHOLINESTERASE LEVEL AMONG INSECTICIDE-EXPOSED VEGETABLE FARMERS IN PREK BALATCHHENG, CAMBODIA: A CASE STUDY.

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ABSTRACT

This study aimed to determine factors affecting cholinesterase (ChE) levels among 153 insecticide-exposed vegetable farmers in one Cambodian village, where 153 factory workers were selected as a comparative non-exposed group. The research instruments were questionnaires and reactive-paper test kits. The study data were analyzed for frequency, percentage, mean, standard deviation, and by using logistic regression. The majority of vegetable farmers were male with approximately 87% of the total participants with an average age of 34 years, where the ages were ranged from 30 to 45 years. The vegetable farmers used a mixture of five different pesticides on the average, i.e., insecticides (Organophosphates and Carbamates, herbicides, and fungicides) for spraying. The estimated personal hygiene scores of vegetable farmers were at moderate level approximately 70.8% among 108 farmers, whereas 85.6 % of the farmers had poor score concerning knowledge of insecticide use among 131 farmers. It was found that 77.8 % of farmers, or 119 farmers, had low abnormal ChE levels. Multiple logistic regression analysis could identify only two factors associated with cholinesterase level, where the period of insecticide spraying was fallen between 30 and 60 minutes with the values of odd ratio (OR) = 6.51, 95% confidence interval (CI) = 1.70 - 24.90, and when the period of spraying took longer than 60 minutes, which the values of OR = 5.04, 95% CI = 1.43 - 17.74. The period of insecticide spraying during the most recent spraying session was carried out between one to three days, with the values of OR = 4.53, 95% CI = 1.62 - 12.69, and the difference was at the 0.004 level (or P-value = 0.004). It was recommended that farmers should avoid exposure to insecticide by reducing insecticide-spraying times, increase their use of personal protective equipment (PPE), and undergo training on insecticide use. These combined measures should improve the insecticide-related health status of vegetable farmers in this area.

Keywords: Cholinesterase level, vegetable farmers, organophosphate and carbamate, reactive-paper test kit

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INTRODUCTION

Insecticides have played a significant role in increasing crop production and ensuring food security worldwide (Jensen et al., 2010; Kamanyire and Karalliedde, 2004; Zou et al., 2007; Yassin et al., 2002). However, many kinds of insecticides used in developing countries do not meet the international standards of the World Health Organization (Zyoud et al., 2010). Especially, farmers lack knowledge of correct personal protective equipment (PPE) use and good personal hygiene (Kachaiyaphum et al., 2010; Jenson et al., 2011).

Farmers can be exposed to insecticide while spraying, and chemicals can enter the body through the gastro-intestinal tract, via inhalation, and through the skin (Casals et al., 2008). Over-exposure to organophosphates (OPs) and carbamates (CMs) can result in acetylcholinesterase (AChE) inhibition, producing an accumulation of acetylcholine (ACh) in the body, with negative effects on health status. While the immediate health-status impacts of both OPs and CMs on cholinesterase inhibition are similar, blood cholinesterase levels return to normal much more quickly after exposure to CMs (Dorko et al., 2011; Pariente et al., 2010).

A wide range of health effects from insecticide poisoning have been reported, especially in developing countries (Lu, 2005), including Cambodia (Jensen et al., 2010). In rural areas of less-developed countries, self-poisoning from organophosphate (OP) insecticides is an important clinical issue, where it kills approximately 200,000 people every year (Rastogi et al., 2010).

Therefore, health screening, by measuring AChE activity in the blood, is a very important method for early detection and diagnosis of OP and CM poisoning. The AChE enzyme and another closely related ChE enzyme are also present in blood, where they provide effective biomarkers for monitoring of OPs and CMs insecticide exposures (Hofmann et al., 2008), and can be used in rapid methods of screening for health effects (Rodríguez-Fuentes and Gold-Bouchot, 2000; Hamers et al., 2000). As the cholinesterase (ChE) enzyme is one biomarker of insecticidal effects, so different instruments have been developed for screening, like the Environmental Quality Management (EQM) (Hofmann et al., 2008) and reactive paper test kits (Kachaiyaphum et al., 2010).

Reactive paper in particular has been used widely in screening farmers exposed to mixed pesticides, for a variety of reasons, i.e., since it is non-invasive and more comfortable for screening in the study site without having to expend time on extensive analysis in the laboratory, and it is not expensive (Kachaiyaphum et al., 2010). While several studies have shown that gender, personal hygiene (Kachaiyaphum et al., 2010), type of insecticides (Klinman et al., 2011) are all potential risk factors linked to ChE enzyme levels (Kachaiyaphum et al., 2010; Pariente et al., 2010), other factors, such as knowledge and practices, are also relevant in explaining levels of occurrence (Klinman et al., 2011).

Although insecticide exposure and health effects have been studied in many countries, there are still a limited number of studies that have examined ChE levels and insecticide use in developing countries. In Cambodia, very few studies were done in relevant to insecticide exposure and ChE level. The objective of this study was to determine factors affecting ChE levels among vegetable farmers, which was exposed to organophosphate and carbamate insecticides.

MATERIALS AND METHODS

Study population

This cross-sectional study used a questionnaire and ChE blood test to elicit information about insecticide use and health effects of insecticides on vegetable farmers in Traeuy Sla Commune in Cambodia.One hundred and fifty-three (153) vegetable farmers were selected in one village to serve as the insecticide-exposed group, while 153 factory workers were selected as a comparative non-exposed group to undergo a ChE blood test (but not complete the questionnaire). The criteria use were vegetable farmers who had been used insecticides at least one month before the research study. Candidate subjects were excluded if they were unwilling to serve as study subjects. All those who agreed to participate were provided written informed consent. The Institutional Review Board (IRB) of Burapha University, and the National Ethics Committee for Human Research in Cambodia, approved the study protocol.

Tools

Questionnaires were used as a tool to interview the study subjects in order to acquire various background information, such as demographic information that included sex, age, and education, health history that included disease and drug use, work history that included type of vegetable, land size, and types of insecticide, and details about personal hygiene that included eating, drinking, and smoking, and knowledge of insecticide use. Scores of personal hygiene were given as four point-rating scales, i.e., always = 4, sometimes = 3, often = 2, and never = 1, while the knowledge score was recorded as yes or no where positive questions were scored as yes = 1 and no = 0, whereas negative questions were scored as yes = 0 and no =1. Scores related to personal hygiene and knowledge were classified into three groups based on Bloom theory, i.e., group 1 = poor where score was $\leq$ 60%, group 2 = moderate where score was >60 - 79%, and group 3 = good/high where score was >80%. Adapting existing compilations from previous studies drew up the questions. The questionnaires were sent to specialists with expertise in related fields in the Faculty of Public Health, Burapha University to check for the validity before use as a data-collection tool. The non-exposed factory-worker group did not complete the questionnaire.

Reactive-paper test kits were used to determine ChE levels among the study and the control groups. The kits were composed of reagent paper, needle, lancet, capillary tubes, glass slides, alcohol, and standard-color paper. Annotation of the product labels after use controlled the results, and the samples were subsequently stored in an icebox to maintain the quality of the paper, due to its sensitivity to elevated temperatures.

Data collection

The questionnaires were translated into the Cambodian language to facilitate the interviewing of local farmers. The chemical reactive-paper test kit, which reacted with the blood and showed colors were used to illustrate the levels of risk. Blood samples were only taken from the participants who expressed permission. Blood tests of the non-exposed factory-worker group were analyzed with the permission of the Department of Occupational Health and Safety, Cambodia.

A general-practice physician of the Department of Occupational Health and Safety, in Cambodia, was participated in the collection of the blood samples. The process was done as follows: cleaned the finger with 70% alcohol, finger stick with a lancet, collected blood in a cap tube (3/4), waited several minutes to allow serum to be separated from red blood cells, decanted from the tube to test serum ChE level with reactive paper in order to determine the exposure and risk against insecticides. The results of the reactive paper test were visualized in four colors, i.e., yellow color means the level was normal or $\geq$ 100 units/ml, yellow-green color means the level was safe or = 87.5-99.9, green color means the level was risky or = 75.0-87.4, whereas blue color means unsafe or < 75.0. The test results were classified into Category A where the color appeared as yellow or yellow-green, which was considered as 'normal' result, while the Category B was considered when the color appeared as green or blue, which was considered as 'abnormal' result.

Data analysis

Data were analyzed using SPSS version 20.0. Descriptive statistics were used to describe percentages, means, and standard deviations, median (min-max). Logistic regression was used to examine possible associations between abnormal ChE level and various factors, i.e., sex, type of vegetable, type of insecticide used, period of insecticide spraying, job function, frequency of spraying, insecticide mixing, last spraying session, knowledge score, and personal hygiene score. The individual variables that showed significant associations were then examined concurrently with multiple logistic regressions. The Chi-squared test was used to determine the significance of differences in ChE levels between the vegetable farmers (exposed) and the factory workers (non-exposed).

RESULTS

Demographic characteristics

It was found that 87 % of vegetable farmers were male, where the average age of the participants was 34 years, and 71.2 % of them were married. The level of education was classified into six levels, i.e., 13.1% were considered as illiterate level, 35.9 % completed primary school, 38.6 % completed secondary school (equivalent to grades 7 to 9), 11.8 % completed high school (equivalent to grades 10 to 12), whereas only 0.7 % obtained bachelor degree. It was found that about 47.7 % of the vegetable farmers had monthly income greater than 1,000,000 riel, 17.6 % of them had monthly income in the range of 800,000 to 1,000,000 riel, with 3.9 % of them was considered as the lowest income group that had monthly income less than 200,000 riel. The average monthly income of the vegetable farmers was 900,000 riel (the rate of exchange was 4,000 riel per $1 USD).

Health histories

It was found that 15.70 % or 24 out of 153 vegetable farmers had experienced some diseases in the past, where 6 of them or 25% had renal diseases, 3 or 12.5% of them had diabetes mellitus, 13 or 54.2% of them had cardiovascular diseases, and 2 or 8.3% of them had both diabetes and cardiovascular diseases. In addition, 6 or 3.9% of them had been used anti-malarial drugs, and 17 or 11.11% of them had been used drugs to treat malnutrition.

Work histories

Results of work histories are summarized in Table 1. It was shown that the majority of participants, about 66 %, preferred to grow only one kind of cabbage, Brassica chinensis. The average land area used was 3,115 m^2, where the land area ranged from 100 to 52,500 m^2. The average number of years, which insecticides had been used was 10.1 years. It was found that 34% of them had been mixed insecticides with an average of 5 different insecticides before spraying, where 44.4 % of them were applied 3 times/week, 39.2 % of them applied 2 times/week, whereas 88.9% of them used the mixture of OPs and CMs insecticides. The average time of spraying session use was 92.6 minutes. It was interesting to note that 63.4% of them sprayed their vegetables within 3 days or less. The average time of recent spraying session was 6 days. It was demonstrated that 32.7 % of them disposed the insecticide containers in the vegetable field, whereas 22.9 % of them burnt the containers.

Table 1. Working histories of vegetable cultivators.

Working histories	Vegetable farmers (N=153)	
	n	%
Type of vegetables		
Brassica chinensis	103	66
Brassica chinensis and salad	24	15.7
Brassica chinensis and eggplant	9	5.9
Other	19	12.4
Land area (m^2)		
≤ 5,000	17	11.1
> 5,000-10,000	131	85.6
> 10,000	5	3.3
Mean (±SD)	3,115 (±4912)	
Median (min-max)	1,920 (100-52,500)	
Duration of insecticide use (years)		
≤ 3	36	23.5
> 3-6	31	20.3
> 6	86	56.2
Mean (±SD)	10.1 (±7.98)	
Median (min-max)	10 (1-33)	
Number of pesticides (insecticide, herbicide, fungicide) mixed before spraying		
≤ 4	51	33.3
> 4-6	87	56.9
>6	15	9.8
Mean (±SD)	5 (±1.3)	
Median (min-max)	5 (2-10)	

Frequency of spraying (time(s)/ week)	
1	9.2
2	39.2
3	44.4
> 3	7.2
Types of insecticide	
Organophosphates	3.3
Carbamates	7.8
Organophosphates and carbamates	88.9
Time for spraying insecticides (minutes)	
≤ 30	12.4
> 30-60	35.3
> 60-90	17
> 90-120	20.9
> 120	14.4
Mean (±SD)	
Median (min-max)	
Most recent spraying session (days)	
≤ 3	63.4
> 3-6	9.2
> 6	27.5
Mean (±SD)	
Median (max-min)	
Insecticide-container disposal	
Discarded into an open field	32.7
Buried	9.2
Burnt	22.9
Put in rubbish and trash	2.6
Discarded into an open field and buried	6.5
Discarded into an open field and burnt	10.5
Others	15.6

Personal hygiene and knowledge

Results of participants using personal protective equipment are summarized in Table 2. It was found that the majority of them (82.4%) used personal protective equipment (PPE), while 66.7% of them wore facemasks, and 31.7% of them wore boots. Only a few (3.2%) wore respirators, and all of the persons who used PPE wore long-sleeved shirts and long trousers. It was also found that a moderate score (70.8 %) was detected for personal hygiene with an average of 5.09. The majority of farmers (85.6%) had low knowledge scores, while only 13.1% of them had moderate scores (see Table 3).

Table 2. Showing personal protective equipment wearing during the application of insecticides.

Type of personal protective equipment	Personal protective wearing	
	Yes (%)	No (%)
Respirator	4 (3.2)	122 (96.8)
Eyeglasses	9 (7.1)	117 (92.9)
Facemask	84 (66.7)	42 (33.3)
Boots	40 (31.7)	86 (68.3)
Others (long sleeve shirt, and long pants)	126 (100)	0.00
Total	126	100

Note: Vegetable farmers could select more than one type of PPE

Table 3. Showing results of personal hygiene and knowledge score on insecticides use.

Personal hygiene/ Knowledge score (%)	Frequency	Percent (%)
Personal hygiene		
High (> 80%)	12	7.8
Moderate (60%-79%)	108	70.6
Low (< 60%)	33	21.6
Knowledge		
High (> 80%)	2	1.3
Moderate (60%-79%)	20	13.1
Low (< 60%)	131	85.6
Total	153	100

Cholinesterase level

It was demonstrated that 17% of participants had unsafe ChE levels, with 60.8% were in the level of risky, while 16.3% of them were within safe margins. The low level of ChE was detected within the lowest part of the normal range (yellow color) in 5.9% of participants, as shown in Table 4. On the other hand, 99.03 % of the control group of factory workers showed normal ChE levels, with only 0.7% of abnormal found in the control group. The Chi-squared test showed the ChE levels of the two groups were statistically significant where the significance level was chosen to be 0.001.

Table 4. Showing result of detected ChE level in vegetable farmers and factory workers.

Reactive paper test kit	Vegetable farmers (N=153)		Factory workers (N=153)	
	n	(%)	n	(%)
Blue (unsafe)	26	17.0	0	0.00
Green (risky)	93	60.8	1	0.7
Yellow-green (safe)	25	16.3	20	13.1
Yellow (normal)	9	5.9	132	86.2

Factors affecting ChE level

It was found that only two factors were found to be significantly associated with cholinesterase level, i.e., the period of insecticide spraying that fell between 30 and 60 minutes where the value of OR was 6.51, 95% CI = 1.70 - 24.90, while the value of OR was 5.04, 95 % CI = 1.43 – 17.74 was observed for the spraying time that was more than 60 minutes, whereas the value of OR was 4.53, 95% CI = 1.62 = 12.69 was observed for the most recent spraying session that took place from three days with the difference was significant at the 0.004 level, as shown in Table 5.

Table 5. Showing results of analyses of factors-sex, type of vegetable, type of insecticides use, period of insecticides spraying, job function, frequency of spraying, insecticides mixing, last spraying session, knowledge score, and personal hygiene score affecting abnormal ChE level by multiple logistic regressions with stepwise methods.

Factors	B	S.E.	Wald (df)	p-value	OR (95%CI)
Period of insecticides spraying (minutes)					
≤ 30	ref				
> 30-60	1.87	.684	7.496 (1)	0.006	6.51 (1.70-24.90)
> 60	1.61	.641	6.373 (1)	0.012	5.04 (1.43-17.74)
Most recent spraying session (days)					
≤ 3	1.51	.525	8.314 (1)	0.004	4.53 (1.62-12.69)
> 3-6	1.49	.919	2.657 (1)	.103	4.47 (0.73-27.13)
> 6	ref.				
Constant	-2.572	1.23	4.333 (1)	0.037	

DISCUSSION

The finding showed that most of the vegetable farmers or 87 % of participants that involved in applying, mixing, and handling insecticides were males. This finding was similar to those reported by Kachaiyaphum et al. (2010), Jenson et al. (2011), and Lu (2005), who all found that most farmers working with insecticides were males. The average age was 34 years (range from 30 - 45 years), as shown in other studies focusing on developing countries, such as Zyoud et al. (2010), who found the largest age group was in the range of 30 - 39 years. Recena et al. (2006) reported that the age group was in the range of 31 - 50 years, which was the largest group (58.4%). The farmers used five kinds of pesticides (OPs, CMs, and others) mixed together on average. However, this was not consistent with the observation of Jenson et al. (2011), who found that the farmers used an average of three types of insecticides.

This study found that vegetable farmers in this given Cambodian village were much more likely to have abnormal cholinesterase levels than those of a comparison group of factory workers. The main factor affecting ChE level was the length of time that they spent spraying insecticide, which was in the range of 30 to 60 minutes where the value of OR was 6.51, 95% CI = 1.70-24.90, whereas the value of OR was 5.04, 95% CI = 1.43-17.74, which was found in those who spent longer time than 60 minutes. This observation was not consistent with results reported by Jensen et al. (2010), who found that numbers of hours spent spraying with OPs or CMs were not a risk factor for the abnormal ChE level with the value of OR was 0.58, 95% CI = -1.95 - 3.12, where the difference was significant at the 0.64 level. In contrary, Kachaiyaphum et al. (2010) found that permanent workers who sprayed more than three times/month were likely to develop low abnormal ChE levels, with the difference was significant at the 0.001 level.

It has been shown that most farmers with low abnormal ChE levels are resulting from recently expose to insecticide, where the half-life of ChE inhibition by OPs is about 30 days (Kachaiyaphum et al., 2010). However, the half-life was shorter than that of ChE inhibition by CMs, which was described by Dorko et al. (2011), who found that the bendiocarbamate was low in toxicity to mammals, and that cholinesterase activity returned to normal within 24 hours after acute exposure. Many researchers have found a variety of factors associated with low abnormal cholinesterase activity, i.e., Magauzi et al. (2011) found that non-use and lack of knowledge were statistically significant, which was associated with abnormal ChE level, where the values of OR was 2.00, 95% CI: 1.07 - 3.68, and OR was 2.02, 95% CI: 1.02-4.03, respectively. Furthermore, Kachaiyaphum et al. (2010) found that the difference of male gender was significant at the 0.003 level, and had discovered a statistically significant of perceived susceptibility associated with abnormal serum cholinesterase level at the 0.001 level.

The present study found no association between ChE level and either personal knowledge or reported hygiene or PPE use. This result was consistent with that reported by Kachaiyaphum et al. (2010), who found that poor knowledge was not related to low abnormal serum ChE level (p-value = 0.26). Jensen et al. (2010) also found that female gender was protective factor against abnormal ChE level (p-value = 0.001). Del Prado-Lu (2007) found that red blood cell (RBC) cholinesterase level was positively associated with age (p = 0.02) and selling the pesticide container (p = 0.008).

The only one limitation that occurred during this study was that there were no questionnaire information for the non-exposed factory workers, because the Ministry of Labor only allowed to use blood ChE enzyme levels as a screening test for the factory workers.

This study supports a number of recommendations. The scores of insecticide-related personal hygiene and knowledge levels of the vegetable farmers were very low. Thus, it is suggesting that training is needed for the farmers. Vegetable farmers should improve personal hygiene, such as using adequate PPE during all insecticide-related activities, to reduce the impact of insecticide exposure. Period of insecticide spraying is a risk factor for abnormal

ChE level. Farmers should reduce the time of insecticide spraying, increase the use of PPE, and undergo training on insecticide use to improve the insecticide-related health status of vegetable farmers in this area.

Further studies should determine ChE levels for at least two times including a baseline measurement before using insecticide, and then during or soon after the cultivation period. Future studies will be stronger if the non-exposed comparison group is more similar to the exposed group (for example, organic farmers). The effectiveness of insecticide-related training to prevent exposure and improve personal hygiene should also be evaluated. The determination of red blood cell cholinesterase with an EQM test kit (EQM Research, Inc.), or laboratory analysis would provide more reliable test results than the rapid screening test used in this study.

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