ความสัมพันธ์ระหว่างการปนเปื้อนออร์แกโนฟอสเฟตในกระแสเลือดของเกษตรกรสวนผักกับความรู้และพฤติกรรมของเกษตรกรในจังหวัดร้อยเอ็ด

ASSOCIATION OF ORGANOPHOSPHATE CONTAMINATION IN BLOOD OF VEGETABLE FARMERS WITH THEIR KNOWLEDGE AND BEHAVIOR IN ROI ET PROVINCE

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บทคัดย่อ

แอซิติลโคลีนเอสเทอเรส (AChE) เป็นเอนไซม์ที่ย่อยสลายสารสื่อประสาทแอซิติลโคลีน ทั้งนี้เพื่อป้องกันการกระตุ้นเส้นประสาทหลังไซแนปส์และกล้ามเนื้อมากเกินไป ออร์แกโนฟอสเฟต (OPPs) เป็นสารยับยั้ง AChE ที่ถูกนำมาใช้เป็นสารเคมีกำจัดศัตรูพืชที่ใช้กันอย่างแพร่หลายในกระบวนการปลูกผัก ดังนั้น OPPs ที่ปนเปื้อนในผักจึงเข้าสู่ร่างกายผู้บริโภคและเป็นพิษต่อระบบประสาทได้ วัตถุประสงค์ของงานวิจัยนี้มี 2 ข้อ คือ 1) เพื่อตรวจวัดระดับ AChE ซึ่งเป็นตัวบ่งชี้ที่ตรงข้ามกับระดับ OPPs ในกระแสเลือด และ 2) เพื่อศึกษาความสัมพันธ์ระหว่างระดับ AChE กับระดับความรู้และพฤติกรรมการใช้สารเคมีกำจัดศัตรูพืชของเกษตรกรสวนผักในหมู่บ้านแห่งหนึ่งในจังหวัดร้อยเอ็ด มีเกษตรกรสวนผักเข้าร่วมโครงการวิจัย 60 คน ทั้งหมดใช้ OPPs แต่มีเพียงร้อยละ 6.5 ผ่านการอบรมการปฏิบัติทางการเกษตรที่ดี (GAP) เกษตรกรส่วนใหญ่มีผลการประเมินระดับความรู้และพฤติกรรมตนเองในการใช้สารเคมีกำจัดศัตรูพืชอยู่ในระดับสูง (ร้อยละ 90.7 และ 81.4 ตามลำดับ) ผู้วิจัยเก็บตัวอย่างเลือดจากปลายนิ้วนางด้วยเข็มเจาะเลือดและหลอดรูเล็ก และวัดระดับ AChE ด้วยกระดาษทดสอบ (วิธีของ Bigg) ผลการวิจัยพบว่า ตัวอย่างเลือด จำนวน 24 ตัวอย่าง มีระดับ AChE อยู่ระดับปกติ ในขณะที่อีก 19 ตัวอย่าง อยู่ในระดับที่ผิดปกติ (มีเกษตรกร 17 ราย ที่ไม่สามารถติดต่อได้ในวันนัดเจาะเลือด) ผู้วิจัยทดสอบความสัมพันธ์ระหว่างระดับความรู้และพฤติกรรมกับระดับ AChE ในกระแสเลือดโดยใช้สถิติเพียร์สันไคสแควร์ ผลวิจัยพบว่า ระดับความรู้และพฤติกรรมไม่มีความสัมพันธ์กับระดับ AChE ในกระแสเลือด (χ^2 = 0.935, df = 3, p = 0.817 และ (χ^2 = 5.303, df = 3, p = 0.151 ตามลำดับ) หลังจากนั้น ผู้วิจัยได้ใช้โปรแกรมการปรับเปลี่ยนพฤติกรรมเกษตรที่มีระดับ AChE ผิดปกติเป็นเวลา 7 วัน ตามเกณฑ์ของ GAP สำหรับอาหารเพาะปลูกและคู่มือการป้องกันอันตรายจากสารเคมีกำจัดศัตรูพืช พบว่าระดับ AChE มีแนวโน้มเข้าสู่ค่าปกติ

คำสำคัญ: เกษตรกรสวนผัก สารเคมีกำจัดศัตรูพืช ออร์แกโนฟอตเฟต เอนไซม์แอซิติลโคลีนเอสเทอเรส

Abstract

Acetylcholinesterase (AChE), an enzyme that breaks down a neurotransmitter acetylcholine, prevents overstimulation of post-synaptic nerves and muscles. Organophosphates (OPPs) are AChE inhibitors widely used as pesticides in vegetable production. Thus, OPP contamination in vegetables entering human blood circulation can be toxic to neurological system. Purposes of this research were divided into 2 folds 1) to determine the level of AChE, an inverse biomarker enzyme for OPPs present in the blood of vegetable farmers and 2) to investigate association between the level of AChE with the level of knowledge and level of behavior on pesticide uses of vegetable farmers in a village of Roi Et province, Thailand. Forty-six subjects who have been working in vegetable farms were recruited. Basic demographic data showed that 100% of subjects used OPPs, but only 6.5% were certified with the Good Agricultural Practice (GAP). Most of them had high level of knowledge (90.7%) and behavior (81.4%) on pesticide uses. Blood samples were collected from ring finger tips with lancets and capillary tubes. A reactive paper finger-blood test (Bigg's method) was used to measure AChE level. Results showed that 24 blood samples had normal level of AChE whereas the other 19 samples were abnormal. Pearson's chi-squared test was further used to determine an association between the levels of knowledge and behavior with the level of AChE. No association neither between the level of knowledge with blood AChE level in vegetable farmers (χ^2 = 0.935, df = 3, p = 0.817) nor between the level of behavior and blood AChE level in vegetable farmers (χ^2 = 5.303, df = 3, p = 0.151) was observed. Lastly, an intervention program was employed to improve the farmer's behavior according to the GAP for Food Crop and Pesticide Hazard Prevention Guidelines for 7 days and was found that their AChE tended to return to the normal level.

Keywords: Vegetable Farmers, Pesticides, Organophosphates, Acetylcholinesterase Enzyme

Introduction

Pesticides are chemicals widely used in agricultural industries. They can be hazardous to health of consumers and farmers as well as environments. In Thailand, the rate of import for agricultural chemicals increases approximately 30% in the last 5 years to around 600,000,000 US$ [1]. Recently, it has been reported that many farmers purchased pesticides without proper training [2]. The most imported pesticides are organophosphates (OPPs) [3].

OPPs kill insects by blocking acetylcholinesterase (AChE), an enzyme that catalyzes hydrolysis of a neurotransmitter acetylcholine, resulting in overstimulation of neuromuscular system. Exposure to OPPS by inhaling, drinking, or ingesting with food is toxic to human body and is depicted by reduced blood AChE level. Acute OPP poisoning symptoms can be ranged from increased salivation, diarrhea, vomiting, muscle tremors, gastrointestinal upset, and confusion [4]. The onset of symptoms is often within

minutes to hours and last for days to weeks. In long term, lower-dose exposure of OPPs has been reported to cause polyneuropathy and cardiovascular diseases [5]. At a national level, the Royal Thai Government has been ardent in monitoring hazardous chemicals by issuing a Hazardous Substance Act B.E. 2535 (1992) amended B.E. 2544 (2001) and B.E. 2551 (2008) in order to regulate the use of chemicals [6]. Pesticide uses in farms are inspected by Good Agricultural Practice (GAP) policy established by the Department of Agriculture of the Ministry of Agriculture and Cooperatives [7]. At a personal level, GAP for fresh fruit and vegetable (FFV) is compulsory for FFV exports to certain countries. However, local FFV farmers are not obliged to be GAP-certified [8]. In other words, local FFV farmers are less controlled and more vulnerable to pesticide toxicity.

Objectives

This research was aimed to investigate the level of AChE in the blood of vegetable farmers and to verify association between the level of AChE with the level of knowledge and behavior on pesticide uses of vegetable farmers in a village of Roi Et province, Thailand.

Methods

Human subjects and research design

Forty-six vegetable farmers voluntarily participated in this research. Inclusion criteria are 1) age $\geq$ 20 years old, and 2) being vegetable farmers. This research used a mixed method of descriptive study and pre-and-post test experiment. All protocols were approved by the Ethics Committee, Faculty of Public Health, Mahasarakham University.

Demographic, knowledge, and behavior data collections

Three questionnaires were used in this study: 1) demographic and personal characteristics data, 2) knowledge on pesticide uses (Table 1), and 3) behavior on pesticide uses (Table 2). These questionnaires were approved for content validity by 3 experts in Nutrition and Occupational Health. They have had over ten-year experiences working with Nutrition and Food Safety. The content validity has been confirmed by index of conjugate (IOC), which was calculated with the following formula.

$$\mathrm{IOC} = \frac{\Sigma R}{N}$$

Where;

$\sum R$ = total scores from the experts

N = number of the experts

Only the items with IOC scores $\geq$ 0.5 were qualified for the questionnaires. The coefficient of reliability is expressed as Cronbach's α calculated as follows.

$$\alpha = \frac{N \cdot c}{V + (N-1) \cdot c}$$

Where;

N = number of items

c = the average inter-item covariance among the items

v = the average variance

The items with Cronbach's $\alpha \geq$ 0.7 were acceptable and used in the questionnaires.

Table 1. Questionnaire of knowledge on pesticide uses

Knowledge on pesticide uses
1. Pesticides should be kept in a confined and safe place.
2. Frequently sprayed pesticides can accumulate in the body.
3. If pesticides are spilled, it should be immediately cleaned with detergent.
4. An empty pesticide container should be destroyed and buried.
5. The higher dose of pesticides used, the higher chance of accumulation in the body.
6. A principle of choosing pesticides is to choose a low poison, but suitable for specific plants.
7. Skin rashes from pesticides can spontaneously disappear.*
8. Pesticides should not be sprayed in the strong wind.
9. Empty pesticide containers can be cleaned and reused for storing food.*
10. Splashes of pesticides into the eyes should be cleaned with a handkerchief.*
11. Pesticides can be mixed together by hands and a paddle.*
12. After spraying pesticides, drink lots of water first before doing other things.*
13. Pesticides should be mixed in a house.*
14. A symbol of hazardous chemicals is a crossed skull on the label.*
15. If a sprayer is clogged, clear it with mouth.*
16. A wool hat cannot prevent a farmer from pesticide sprays.*

*The score of negative questions were reversed. Each item was scored 1 point. The scores (maximally 16 points) of an individual sheet of the questionnaire knowledge on pesticide uses were ranked into 3 levels as follows: Low level = 0-5 points, Medium level = 6-10 points, and High level = 11-16 points

Table 2. Questionnaires of behavior on pesticide uses

Behavior on pesticide uses
1. You mix pesticides with the concentrations indicated on the label.
2. You make sure that the gloves are not leak before use.
3. You mix pesticides with a paddle, not hands.
4. You cleanse your hands with soap after mixing pesticides.

Table 2. (Continued)

Behavior on pesticide uses
5. You prevent yourself, food, drinking water, and other surroundings from the sprayed pesticides.
6. You check your apparatuses before spraying.
7. You read a label before use.
8. You check the wind direction before spraying.
9. You wear a long-sleeve shirt, long trousers, closed shoes, and a mask before spraying.
10. You clean the spraying suits separately.
11. You immediately take a bath after spraying.
12. You wear boots while spraying.
13. You wear long gloves while spraying.
14. You keep spraying tools separately away from other non-contaminated tools.
15. You choose the pesticides with a proper labeling, caution, name of manufacturer, and registered number.
16. You use eyewear to protect your eyes and nose.
17. When you have physical symptoms due to pesticides, you let it spontaneously recover.*
18. When you have physical symptoms due to pesticides, you buy medicines from drug stores.*
19. When you have physical symptoms due to pesticides, you see the doctor.

*The score of negative questions were reversed. Each item was scored using the Likert's scale: Always, 3 points; Sometimes, 2 points; and Never, 1 point. Thus, the possible minimal and maximal scores were 19 and 57, respectively. Subsequently, these scores were ranked into 3 levels as follows: Never practice = 19-31 points, Sometimes practice = 32-44 points and Always practice = 45-57 points

Blood sampling and cholinesterase level measurements

To detect AChE in the body from exposure to OPPs, reactive paper test kit was purchased from The Government Pharmaceutical Organization, Ministry of Public Health, Thailand. Blood samples were taken by public health officers in the area. The paper color changes were compared with the standard and divided in 4 levels of AChE as shown in Table 3 [9].

Table 3. Reactive paper color interpretation

Reactive paper color	AChE level (units/ml)	Health status
Yellow	≥ 100	Normal
Yellow-Green	87.5-99.9	Safe
Green	75.0-87.4	Risky
Blue	<75.0	Unsafe

AChE levels were further categorized into 2 groups. AChE values of ≥ 87.5 units/ml and <87.5 units/ml were considered normal and abnormal, respectively. Samples were collected from August-December 2017, the harvesting and spraying period. In general, farmers spray their vegetables about a week before harvest.

OPP measurement in vegetables

OPPs were measured with the GT-pesticide residual test kit (G9 Co., Ltd., Bangkok, Thailand). Six samples of vegetables collected from the farms were chopped. Five grams of each homogenous sample was transferred to a sample bottle. OPP detection was performed following the procedure described in the instruction leaflet.

Statistical analyses

Data were analyzed with SPSS packaging program (version 20). Descriptive statistics used in this study were mean, standard deviation, and percentage. Chi-square test was to analyze association between level of knowledge and behavior with AChE level.

Results

Majority of local vegetable farmers without GAP certification

In part-1 of the study, a demographic questionnaire was responded by 46 farmers and found that all of them have been using OPPs (100%). However, only 3 farmers (6.5%) were GAP-certified.

Table 4. Demographic Data

Demographic data	Number	Percentage
Gender		
Male	20	43.5
Female	26	56.5
Age (years)		
44-52	8	17.4
53-61	17	37.0
62-70	13	28.9
71-80	8	17.4

Table 4. (Continued)

Demographic data	Number	Percentage
Marital Status		
Married	43	93.5
Single	1	2.2
Divorced/Separated	0	0
Widowed	2	4.3
Educational Level		
Not attended school	0	0
Primary school	34	73.0
Secondary school	3	6.5
High school	4	8.7
Diploma	2	4.3
Bachelor	3	6.5
>Bachelor	0	0
Length of Being Farmers (years)		
<20	5	10.9
20 or more	41	89.1
Averaged Family Monthly Income (baht)		
<5,000	21	45.7
5,000-10,000	18	39.1
10,001-15,000	4	8.6
>15,000	3	6.5
Attended Pesticide Handling Workshop		
Yes	19	41.3
No	27	58.7
Certified Good Agricultural Practice (GAP)		
Yes	3	6.5
No	43	93.5
Using OPPs	46	100.0

No association between the level of knowledge on pesticide uses with blood AChE level in vegetable farmers

In part-2, a questionnaire testing for the level of knowledge on pesticide uses. As shown in Table 5, 90.7% of the farmers had a high level of knowledge on pesticide uses. Meanwhile, 43 blood samples were collected for AChE level measurements (3 subjects were dropped out due to their personal works). It was found that most of the subjects had a high level of knowledge onpesticide uses. Then we calculated Chi-square test for knowledge-AChE association. Result showed that there was no association between the level of knowledge and blood AChE level in vegetable farmers (χ^2 = 0.935, df = 3, p = 0.817, Table 6).

Table 5. Level of knowledge on pesticide uses

Level of knowledge	Number	Percentage
High (11-16)	39	90.7
Medium (6-10)	4	9.3
Low (0-5)	0	0
Total	43	100.0
Mean = 13.67, S.D. = 2.13, Max. = 16, Min. = 9		

Table 6. Association between level of knowledge on pesticide uses and blood AChE level

Level of Knowledge	Blood AChE level				Total
	Normal (%)	Safe (%)	Risk (%)	Unsafe (%)	
High	6 (14.0)	15 (34.9)	12 (27.9)	6 (14.0)	39 (90.7)
Medium	1 (2.3)	2 (4.6)	1 (2.3)	0	4 (9.3)
Low	0	0	0	0	0
Total	7 (16.3)	17 (39.5)	13 (30.2)	6 (14.0)	43 (100.0)

No association between the level of behavior on pesticide uses with the blood AChE level in vegetable farmers

Similarly to the level of knowledge, 81.4% of the farmers had a high level of behavior on pesticide uses (Table 7). Again, there was no association between levels of behaviors and blood AChE level in vegetable farmers (χ^2 = 5.303, df = 3, p = 0.151).

Table 7. Level of behavior on pesticide uses

Level of behavior	Number	Percentage
High (11-16)	35	81.4
Medium (6-10)	8	18.6
Low (0-5)	0	0
Total	43	100.0
Mean = 46.83, S.D. = 3.57, Max. = 53, Min. = 38		

Table 8. Association between level of behavior on pesticide uses and blood AChE level

Level of Behaviors	Blood AChE level				Total
	Normal (%)	Safe (%)	Risk (%)	Unsafe (%)	
High	6 (14.0)	16 (37.2)	8 (18.6)	5 (11.6)	35 (81.4)
Medium	1 (2.3)	1 (2.3)	5 (11.6)	1 (2.3)	8 (18.6)
Low	0	0	0	0	0
Total	7 (16.3)	17 (39.5)	13 (30.2)	6 (14.0)	43 (100.0)

Behavioral modification on blood AChE level in vegetable farmers In part-3, the level of AChE in 46 samples was then divided into 2 groups as described in Materials and Methods: normal (24 samples) and abnormal (19 samples) (Table 9). Subsequently, 19 farmers with the abnormal AChE level underwent an intervention program to improve their behavior according to the GAP for Food Crop [10] and Pesticide Hazard Prevention Guidelines (Department of Disease Control) for 7 days. Blood samples were collected and determined for AChE level measurements again (Table 9). Results showed that number of subjects with normal AChE level increased from 24 to 27.

Table 9. AChE level in vegetable farmers before and after interventions

AChE level	Number	Percentage
Before interventions		
Normal	24	55.8
Abnormal	19	44.2
Total	43	100.0
After interventions		
Normal	27	63.8
Abnormal	16	36.2
Total	43	100.0

Conclusions and Discussion

The finding that only 6.5% of the farmers were GAP-certified implies that the farmers might not follow a safety guide of pesticide uses as they self-reported. Food safety is a crucial issue for food exporters in Thailand. In the year 2004, there has been the "Roadmap of Food Safety" which consists of 5 strategies: 1) production inputs and raw materials, 2) farm production, 3) food processing, 4) agricultural products, and 5) marketing [11]. Therefore, GAP is an essential element in the roadmap of food safety. The exporting farmers who achieve the qualification of the national GAP criteria can stamp their products with GAP sticker. However, a lack of confidence in food safety of FFVs in the domestic markets still exists [8].

Our findings showed that there was no association between blood AChE level and the level of knowledge of the farmers on both behavior on pesticide use domains, which were different from the study of Namwong and coworkers. Namwong; et al. reported that farm workers with low level of knowledge had a concordant low-level AChE [12]. Further investigation is required to clarify this discrepancy.

As the behavioral modification with the GAP for Food Crop and Pesticide Hazard Prevention Guidelines for 7 days improved the blood AChE level, this modification method should be applied to a larger group of farmers. Indeed, it has been already reported that hand washing and showering immediately after exposure to pesticides were enough to reduce bioaccumulation of toxic substances in the body [6]. Importantly, farmers should be encouraged to follow the GAP requirements (item 3 in the table 1) for their safety [10].

In conclusion, the level of knowledge and behavior is not associated with the level of blood AChE. Even though the farmers reported the high levels of knowledge and behavior, their AChE level was not as high as expected. Other strategies might be needed to solve this problem.

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