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Blood cholinesterase activity levels of farmers in winter and hot season of Mae Taeng District, Chiang Mai Province, Thailand

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Abstract Organophosphate and carbamate pesticides have been widely used by farmers for crop protection and pest control. Inhibition of acetylcholinesterase (AChE) in erythrocyte and butyrylcholinesterase (BChE) in plasma is the predominant toxic effect of organophosphate and carbamate pesticides. Mae Taeng District, Chiang Mai Province, is one of the large areas of growing vegetables and fruits. Due to their regular exposure to these pesticides, the farmers are affected by this toxicity. The objective of the study was to examine the AChE and the BChE activity levels in the blood of 102 farmers for comparison of exposure in two cropping seasons, winter and hot. Blood samples were collected in December 2013 (winter) and April–June 2014 (hot). A total of 102 farmers joined the study, represented by 76 males (74.5 %) and 26 females (25.5 %). The age of most of the farmers was 53.4±8.7 years. Out of 102, 21 farmers used carbamate pesticides. The results showed that the AChE and the BChE activity levels of all the farmers were 3.27±0.84 Unit/mL and 2.15±0.58 Unit/mL, respectively. The AChE and the BChE activity levels in males were 3.31±0.88 Unit/mL and 1.97±0.60 U/mL, respectively, during winter and 3.27±0.82 Unit/mL and 2.15±0.58 U/mL, respectively, during the hot season, and AChE and the BChE activity levels in females were 3.27±0.82 U/mL and 2.44±0.56 U/mL, respectively, during the hot season. The cholinesterase activity levels, both AChE and BChE, in the male farmers' blood had significant difference between the two seasons, while in the case of the female farmers, there was significant difference in the BChE activity levels, at $p < 0.05$. The BChE activity level was found to significantly correlate with self-spray ($p < 0.05$), which implies that the BChE activity decreased when they sprayed by themselves. The cholinesterase activity levels of the present study were lower than those of the other studies, which may be an indication of some chronic effect of exposure to anticholinesterase pesticides. Thus, it is recommended that the use of pesticides be decreased, together with increase in the awareness of the impact of pesticides on health; also recommended is regular monitoring of blood cholinesterase.

Keywords Cholinesterase · AChE · BChE · Farmers · Chiang Mai Province · Pesticides

Introduction

Organophosphates (OPs) and carbamates, used in crop protection, may produce toxicity to humans by inhibiting AChE activity (Mwila et al. 2013), which would terminate the action of the neurotransmitter acetylcholine (ACh) within nerve synapses, thus resulting in the destruction of AChE (Halbrook et al. 1992).

Blood cholinesterases have been widely used as biomarkers for the monitoring of exposure to OPs and carbamate

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pesticides (Hofmann et al. 2010). There are two cholinesterase enzymes in human body: (1) acetylcholinesterase (AChE, EC 3.1.1.7) present in the nerve system, erythrocytes, brain and several tissues, and (2) butyrylcholinesterase (BChE, EC 3.1.1.8) found in serum or plasma which is synthesized by the liver.

Previous reports have demonstrated the negative impacts of OP-modified active sites on human health. The OPs and the carbamates have high correlations with symptoms and poisoning after exposure. The AChE and the BChE activities have been found to significantly reduce among those humans who had direct or indirect exposure to OPs and the carbamates (Chakraborty et al. 2009; Coye et al. 1986; Makrides et al. 2005; Roldan-Tapia et al. 2006). For this reason, the measurements of AChE and BChE activities have been considered good indicators for assessing the extent of exposure to the OPs and the carbamates among farmers. Various studies on the cholinesterase activity among Thai people have been reported, such as the study conducted by Pongraveevongsa and Ruangyuttikarn (2000) who reported normal range of BChE activity in the serum of blood donors at Maharaj-Nakorn Chiang Mai hospital, Thailand, that is, activity levels of 4.32–6.52 U/mL and 4.88–7.47 U/mL, respectively, in males and females.

Mae Taeng District (Fig. 1) which is located in Chiang Mai Province in the northern part of Thailand has a population amounting to 53,348 in 11 sub-districts. The district has the Ping River, Ngad River, and Taeng River as the main water resources and fertile soil, which enable farmers to grow crops year round. Therefore, Mae Taeng is the important area where high-quality fruits and vegetables are produced for commercial markets. The main plants of cultivation in the area include rice, soya bean, soil bean, garlic, tobacco, sugarcane, longan, litchi, etc. Not surprisingly, most people in Mae Taeng are farmers or perform agriculture-related occupations, and large amounts of OPs and carbamates are applied in the agricultural areas for the purpose of pest control. The present study was conducted to compare the exposure to OPs and carbamates in two

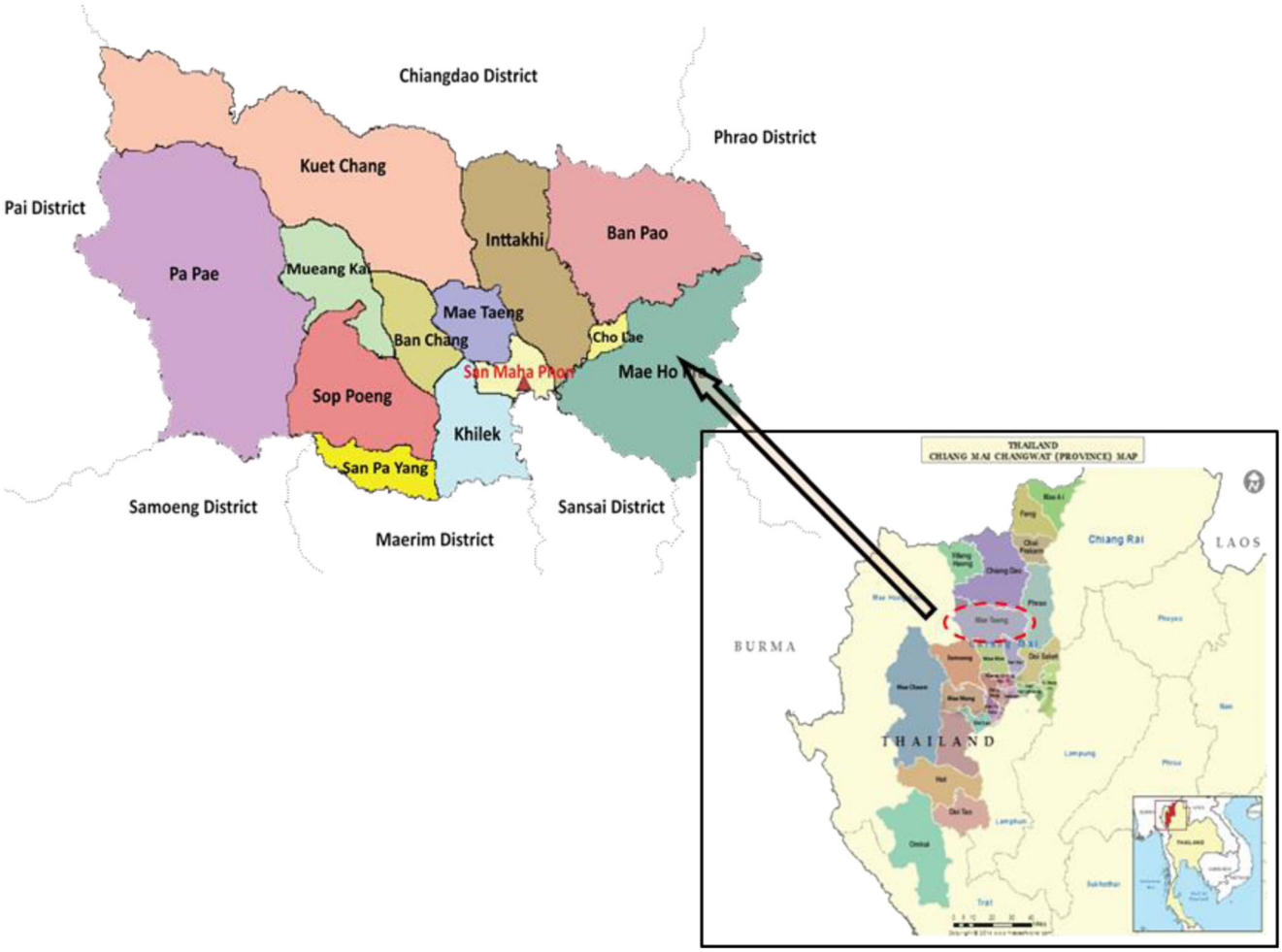

Fig. 1 The map of Mae Taeng District, Chiang Mai Province, Northern Thailand

Table 1 Demographical data of farmers

Data	Results
Number, *n* (%)	102
Male	76 (74.5 %)
Female	26 (25.5 %)
Mean age (year, mean±SD)	53.4±8.7
Male	52.2±9.3
Female	57.1±5.4
BMI (kg/m^2, mean±SD)	
Winter season	24.15±3.8
Male	23.5±3.2
Female	26.0±4.7
Hot season	24.5±3.8
Male	23.9±3.3
Female	26.3±4.8
Years of working in agricultural field, *n* (%)	
1–10	8 (7.8 %)
Male	8 (7.8 %)
Female	0 (0.0 %)
11–20	19 (18.6 %)
Male	13 (12.8 %)
Female	6 (5.9 %)
21–30	33 (32.4 %)
Male	24 (23.5 %)
Female	9 (8.8 %)
>30	42 (41.2 %)
Male	31 (30.4 %)
Female	11 (10.8 %)
Pesticides used by farmers (yes, *n* (%))	
Winter season	74 (72.5 %)
Male	58 (56.9 %)
Female	16 (15.7 %)
Hot season	67 (65.7 %)
Male	51 (50.0 %)
Female	16 (15.7 %)

cropping seasons, winter and hot season, of farmers in Mae Taeng District, Chiang Mai Province, Thailand, by using the activities of plasma BChE and erythrocyte AChE as exposure indexes.

Materials and methods

Chemicals and reagents

Acetylthiocholine iodide (ATCI), s-Butyrylthiocholine iodide (BTCI), and 5,5′-dithio-bis-2-2-nitrobenzoic acid (DTNB), products of Sigma-Aldrich (Steinheim, Germany), were used in the study.

Subjects

One hundred and two farmers from 11 sub-districts of Mae Taeng District, Chiang Mai Province, Thailand, were enrolled as volunteers. The subjects were those who identified themselves with working on crop production and had ages in the range of 18–65 years. Five milliliters of venous blood was collected by using K-heparin as the anticoagulant and transferred using cool containers to Toxicology Laboratory, Research Institute for Health Science, Chiang Mai University. The collection of samples was conducted twice, that is, in December 2013 of 118 farmers (winter season) and during April–June 2014 of 102 farmers (hot season) for the comparison of exposure levels in two cropping seasons, winter and hot season. Hence, the data are presented as regards the number of subjects as being the data of 102 farmers.

The blood samples were centrifuged at 800*g* for 15 min to achieve plasma separation. The plasma sample was transferred to a 1.5 mL microcentrifuge tube. The erythrocyte sample was washed two times using phosphate buffer saline (PBS), pH 7.4, and then centrifuged. The washed erythrocyte sample was transferred to a new 1.5 mL microcentrifuge tube. Both the plasma and the erythrocyte were stored at −20 °C in a freezer until the AChE and BChE analysis.

Cholinesterase activities by Ellman's assay

The AChE and the BChE activities were analyzed in compliance with the method of Ellman et al. (1961) and Gonzalez et al. (2012), with some modification. The cholinesterase activity was measured by using ATCI

Table 2 Comparison of mean AChE and BChE activities of farmers in Mae Taeng District, Chiang Mai Province between two crop seasons (winter= December 2013 and hot season=April–June 2014)

	Male (*n*=76)		Female (*n*=26)		Total (*n*=102)	
	Winter	Hot	Winter	Hot	Winter	Hot
AChE activity, U/mL	4.50±0.73a	3.28±0.86a	3.31±0.88	3.27±0.82	3.31±0.88	3.27±0.82
BChE activity, U/mL	1.92±0.56b	2.05±0.55b	2.12±0.68c	2.44±0.56c	1.97±0.60d	2.15±0.58d

"a," "b," "c," and "d" denote significant difference, by ANOVA, at $p < 0.05$

Table 3 Comparison of mean AChE and BChE activities of farmers in Mae Taeng District, Chiang Mai Province, affected by organophosphate and carbamate insecticide use

Pesticide use	AChE activity, U/mL		BChE activity, U/mL	
	Winter season	Hot season	Winter season	Hot season
Farmers who did not use carbamate and OPs (n=68)	3.55±0.83	3.32±0.94	1.91±0.56	2.15±0.56
Farmers who used carbamate (n=21)	3.29±0.45	3.19±0.48	2.07±0.62	2.20±0.51
Farmers who used OPs (n=7)	3.36±0.62	3.04±0.98	2.01±0.75	1.96±0.72
Farmers who used OPs and carbamate (n=6)	2.97±1.01	3.38±0.55	2.23±0.82	2.24±0.89

and BTCI as substrates for the AChE activity in erythrocyte and the BChE activity in plasma, respectively, and reported as unit per milliliter (U/mL). The results of the AChE and the BChE activities were divided into three categories, namely, normal poisoning, mild poisoning, and moderate poisoning, and presented as % decline in the AChE and the BChE activities, at <20 %, 20–40 %, and >40 %, respectively (Khan et al. 2010).

Ethical considerations

The present study was approved by the Human Experimentation Committee, Research Institute for Health Science (RIHES), Chiang Mai University, Chiang Mai, Thailand (Project No. 9/56). The informed consent was obtained from all the participating farmers. All the data were kept in a confidential manner, and the results reported individually to the participating farmers; the overall data were reported to the public.

Statistical analysis

SPSS version15 was used for the statistical analysis. The cholinesterase, both AChE and BChE, activities were reported in terms of mean, standard deviation. The mean comparison of the AChE and the BChE activities between the seasons, pesticide uses, and percentages of depression were analyzed by ANOVA. The significance level was set at 0.05.

Results

The demographic data of 102 farmer subjects are as shown in Table 1. The subjects included 76 males (74.5 %) and 26 females (25.5 %). The mean age of the farmers was 53.4 years. The females had higher BMI than the males in both the seasons. Most of the famers had been working in the agricultural field for more than 30 years and used pesticides for crop production.

The AChE and the BChE activities of the farmers are presented in Table 2. The activities of AChE and BChE can be used as biomarkers of the OPs and the carbamate effect. In the winter season, the means of the AChE and the BChE activities were 4.50±0.73 U/mL and 1.97±0.60 U/mL, respectively, while in the hot season, these were 3.31±0.88 U/mL and 2.15±0.58 U/mL, respectively. The mean AChE and BChE activities among the male farmers differed significantly between the two seasons. The mean BChE activities of the female farmers showed significant difference between the two seasons, while in the case of the AChE activities, there was no significant difference.

Table 3 shows no significant differences in the AChE and the BChE activities between farmers who used OPs and carbamates and farmers who did not use OPs and carbamates, according to the results of comparison (Table 3). The results show that there was no significant difference.

Discussion

The activities of AChE and BChE can be used as biomarkers of exposure to OPs and carbamates. Those farmers who were exposed to OPs and carbamates showed lower levels of activities of the cholinesterase enzymes (Areekul et al. 1981). With reference to the

Table 4 Percent of decline in AChE and BChE activities among farmers in hot season compared to winter season

Percent of decline	Number of subjects (%)		
	Normal poisoning, <20 %	Mild poisoning, 20–40 %	Moderate poisoning, >40 %
AChE	77 (75.5 %)	14 (13.7 %)	11 (10.8 %)
BChE	98 (96.1 %)	4 (3.9 %)	–

Table 5 Comparison of AChE and BChE levels and agriculture occupational periods

Agriculture occupational period	AChE (U/mL)		BChE (U/mL)	
	Winter season Mean±SD	Hot season Mean±SD	Winter season Mean±SD	Hot season Mean±SD
1–10, (n=8)	3.93±0.79a,b	3.35±.44	1.72±0.44	1.89±0.39
10–20, (n=19)	3.00±0.79a	3.18±0.69	2.00±0.58	2.00±0.50
20–30, (n=33)	3.50±0.51	3.20±0.75	2.05±0.58	2.33±0.54
over 30, (n=42)	3.52±0.79b	3.36±1.03	1.94±0.65	2.13±0.64

"a" and "b" denote significant difference, by ANOVA, at $p < 0.05$

normal range of cholinesterase, the parameter has not been set in the case of Thai people; hence, the present study would have to be compared with previous studies conducted on the same race. The AChE activity analysis performed in the present study examined the level of AChE in the farmers, and it showed that the level was lower than that reported by Chan et al. (1994) who carried out a study on blood donors as normal subjects in Malaysia in which it was found to be in the range of 3.50–13.94 U/mL in males and 6.67–13.16 U/mL in females, and the BChE activity was found to be in the range of 1.02–4.95 U/mL for males and 1.18–4.02 U/mL for females. The present study reported the BChE activity to be a bit higher than that reported earlier (Chan et al. 1994). A comparison of the BChE activities of the present study with those of the study by Pongraveevongsa and Ruangyuttikarn (2000) found that the activity was lower, which may be explained by the difference in the groups of subjects; however, the result supported this study which reported that farmers had lower levels of BChE than people who were engaged in other occupations.

The farmers involved in pesticide application on crops during production were identified and categorized as suffering from normal, mild, and moderate levels of poisoning with % decline in ChE levels in winter season and hot season (Table 4). There was 10.8 % of farmers who had more than 40 % decline in the AChE activity (moderate poisoning) and 75.5 % of the farmers had less than 20 % decline (normal). There were no subjects who had 40 % decline in the BChE activity, and most of the subjects (96.1 %) had less than 20 % decline. Most of the farmers had less than 20 % decline in both the AChE and the BChE levels, which means that the farmers were exposed to the OPs and the carbamates equally in the two seasons of studies, except for some farmers who showed decline in the AChE levels of more than 40 %. Those farmers who showed more than 40 % decline in the AChE levels may have behaved differently in that they must have used the OPs and the carbamates shortly before the collection of the blood samples, and their levels of AChE must have been acutely affected after the use of the OPs and the carbamates.

The results from Table 5 present a pattern of cholinesterase depression during the time of working in the agricultural fields. The lowest mean level of AChE activity can be seen as occurring in 10–20 years of winter and 10–20 years of hot season. Clearly, BChE has the lowest activity in 1–10 years in both the seasons.

The present study reported a significant decrease in the activity of BChE of farmers who had used OP or carbamate insecticide sprays in the previous 6 months in comparison with farmers who had not used such sprays (Table 6). The results obtained are similar to the findings of previous publications (Innes et al. 1990; Ames et al. 1989; Lander and Hinke 1992). Thus, it is evident that exposure to OP and carbamate insecticides might have an effect on the accumulation of ACh at the synaptic junctions, or that it might lead to cytotoxicity (Bardin et al. 1994).

Table 6 AChE and BChE activities of farmers subjected to pesticide spraying activity for previous 6 months

Spray	Season	Winter		Hot	
		No (n=59) Mean±SD	Yes (n=43) Mean±SD	No (n=43) Mean±SD	Yes (n=47) Mean±SD
Acetylcholinesterase, U/mL		3.40±0.80	3.52±0.80	3.25±0.86	3.30±0.83
Butyrylcholinesterase, U/mL		2.05±0.67	1.86±0.47	2.28±0.62a	2.00±0.50a

"a" denotes significant difference, by ANOVA, at $p < 0.05$

Conclusions

The farmers of the present study had low levels of AChE activity in comparison with normal subjects from a previous study, while the BChE activity levels were higher than those of the subjects studied previously (Chan et al. 1994). The BChE activity levels of the farmers were lower than those of normal blood donor subjects from Chiang Mai, Thailand (Pongraveevongsa and Ruangyuttikarn 2000), which may be because farmers have much higher exposure to OPs and carbamates than people of most other occupations. Therefore, the recommendations include decrease in the use of pesticides together with raising of awareness levels regarding the impact of pesticide use on health, coupled with monitoring of the blood cholinesterase levels.

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