Hematological Indices of Pesticide Exposure on Rice Farmers in Southern Thailand

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Abstract

The most common pesticides used in rice farming are organophosphates and carbamates. These pesticides inhibit acetylcholinesterase (AChE) activity, resulting in excessive levels of acetylcholine accumulation and disrupted neurotransmission. This study compared AChE activity and hematological parameters between pesticide-using rice farmers, non-pesticide-using rice farmers, and non-agricultural occupational groups (non-farmers). Pesticide residues that accumulated in the rice, water, and soil of the study area were also determined. AChE activity of all participants showed 10 out of 87 samples (11.49%) as borderline depressed, with 50% of those being pesticide-using farmers. Most of the hematological parameters were normal in all samples. However, platelet numbers of self-spraying pesticide rice farmers were significantly lower than those of non-self-spraying rice farmers. The results suggested that the use of pesticides during rice farming affected AChE activity and some hematological cells. Besides, pesticide residues in soil, water, and rice in the fields using pesticides were higher than in non-pesticide use areas. Results confirmed that the farmers using pesticides experienced higher exposure rates than farmers who did not use pesticides.

Keywords: Acetylcholinesterase, hematological indices, pesticide, rice farmer

Introduction

The main agricultural occupation in Asia is rice farming. For Thailand, approximately 40.9% of the total land area is used for agricultural production, with 31.3% and 27.8% as forest and unclassified land, respectively. Approximately, 49.8% of the agricultural land is used for growing rice; 21.5% for field crops, 21.2% for fruit or horticultural crops, and 7.5% for others. Thailand is nearly self-sufficient in terms of food production; agriculture is an important sector and is the largest source of employment for the rural population. Approximately, 46.6% of the total population is engaged in the agricultural sector.¹

Chemicals are used in agriculture to promote high production yields, with several types used as pesticides. The major groups of synthetic organic pesticides are or-

ganophosphates, carbamates, and pyrethroids. Most of these are classified as class III, slightly hazardous, with over 2,000 mg/kg body weight Lethal Doses (LD50) for rats by both oral and dermal exposure.² Although most pesticides are legally allowed chemicals, their use may be harmful to farmers, consumers, and the environment if they are not handled properly with appropriate precautionary methods.

The organophosphate and carbamate pesticides are neurotoxic via acetylcholinesterase (AChE) inhibition, with AChE unable to bind acetylcholine to terminate synaptic transmission. Inhibition of AChE in humans can cause many acute symptoms including dizziness, nausea, difficulty in breathing, and even death. In addition, a non-specific effect results in induced reactive oxygen species that attacks lipids, proteins, and deoxyribonucleic

How to Cite: Kwanhian W, Yimthiang S, Jawjit S, Mahaboon J, Thirarattanasunthon P, Vattanasit U. Hematological indices of pesticide exposure on rice farmers in Southern Thailand. *Kesmas: National Public Health Journal*. 2019; 14(1): 37-42. (doi: 10.21109/kesmas.v14i1.2812)

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Received : December 15th 2018
Revised : May 10th 2019
Accepted : June 12th 2019

acid (DNA), causing oxidation and membrane damage, enzyme inactivation, DNA damage, and cell death.³ Several authors have investigated the adverse hematological effects of organophosphate on blood hemoglobin, hematocrit level, red blood cell numbers, platelet numbers, and white blood cell numbers.^{4, 5}

Because of these known adverse effects of pesticides, many studies have been done. Results suggest that Thai agricultural workers are exposed to pesticides.⁶⁻⁹ However, there not many studies have examined the effects of pesticide use on hematological indices in rice farmers, particularly in Southern Thailand. To provide farmers with a better quality of life, surveillance for poisoning caused by occupational pesticide exposure is necessary. Therefore, the major aim of this study was to investigate the red blood cell AChE and hematological status of rice farmers in Southern Thailand that employed pesticides during the planting process, compared to non-pesticide-using farmers and non-farmers as control groups.

Method

The selected areas of the present study were Pak Phanang Fang Tawan Tok, Pak Phanang, and Nakhon Si Thammarat, Thailand. The earlier studies reported that the pesticides used in this area were mainly organophosphates and carbamate. It is also reported that the top three pesticides for which residues were found to contaminate rice fields and rice grain were parathion methyl, fipronil, and chlorpropham, respectively.¹⁰ Population sample size was calculated using the Yamane formula with 0.1 degrees of error from a population of 5,711 individuals. The total number of sample should not be less than 98. In this study, there were 102 participants comprised of 37 pesticide-using rice farmers, 31 non-pesticide-using rice farmers, and 34 non-farmers (non-agricultural occupation). The samples were selected using a systematic sampling method. Inclusion criteria for pesticide-using rice farmers consisted of rice farmers aged between 18 and 60 years who used pesticides in their cultivation processes. For non-pesticide-using rice farmers, the inclusion criteria was rice farmers in the same 18–60-year age range who did not use pesticides in their cultivation processes. For the non-agricultural occupational samples, the inclusion criteria were defined as the same 18–60-year age range for people whose occupation was not involved with agriculture. Individuals with records of diseases, including various cancers and hematologic, respiratory, cardiovascular, gastrointestinal, and endocrine system illnesses, were excluded.

Certified nurses or medical technologists drew six milliliters of blood from veins in participants' arms and split it into two Ethylenediaminetetraacetic acid (EDTA) collecting tubes. Demographic data and information on pe-

sticide use were collected by researchers and trained interviewers. There were 50 questions asked, which required approximately 30 minutes per interview. Most questions were either yes or no, dichotomous types or multiple choice, offering several fixed alternatives. In addition to inquiring about demographic characteristics, cultivation methods, and methods of mixing, application, and storage of pesticides, as well as toxicological side effects, the questionnaire inquired about the varieties of pesticides used, and the participants' knowledge about pesticides, attitude, and practices. The questionnaire and this study were reviewed and authorized by the Human Research Ethics Committee, Walailak University, Thailand (WU_010/2017).

AChE activity was determined by a modified electro-metric method.¹¹ In brief, 3 ml of distilled water was placed in a 10 ml beaker; then 3 ml of barbital phosphate buffer was added, followed by 0.2 ml of blood. The pH of the mixture was measured just after the substrate as 0.1 ml of 27.5 mM acetylcholine iodide was added, and the mixture was then placed in a water bath at 37°C for 20 min before the pH was re-measured. The activity was calculated as the change in pH over the 20 min ($\Delta\text{pH}/20$ min). If ΔpH was higher than 1.15, the AChE activity was normal. A ΔpH value between 1.0–1.15 indicated borderline depressed AChE activity, while ΔpH lower than 1.0 indicated severely depressed AChE activity.

Blood was analyzed using an electrical impedance automatic cell counter (Beckman Coulter) for parameters including hemoglobin (Hb), hematocrit (Hct), white blood cell count (WBC), percentage of WBC differentiation (neutrophil, lymphocyte, monocyte, eosinophil, and basophil), and platelet count. The 24 soil and 19 water samples from rice fields were collected from the study area in Nakhon Si Thammarat Province. The soil was collected following the manual instructions for soil sampling and soil analysis methods.¹² Water was collected following standard methods for the examination of water and wastewater.¹³ Unmilled rice was collected from four pesticide and eight non-pesticide-using rice fields.

A quantitative determination commercial, GT-Pesticide Residual test kit was used to determine pesticide contamination in the soil, water, and rice samples. The kit utilizes an AChE inhibition technique by observing the color changes. Organophosphate and/or carbamate are pesticides that interfere with the AChE enzyme activity. The detection limit is 0.05 mg/kg pesticide.¹⁴

One-way analysis of variance (ANOVA) was used to assess differences of the data for more than two sample groups (age of participants), and the independent t-test was used for two independent groups (red blood cell AChE activity and hematological parameters). Statistical analysis was performed using SPSS software Version 13.0 (IBM Corp., Armonk, NY, USA).

Results

The 102 sample participants were divided into three groups which were 37 pesticide-using rice farmers, 31 non-pesticide-using rice farmers, and 34 non-farmers (other occupation). There were 49 males and 53 females with an average age of 53.5 years (Table 1). Only 87 blood samples were collected from the participants consisting of 34 pesticide-using rice farmers, 26 non-pesticide-using rice farmers, and 27 non-farmers. Most participants were older than 40 years, with no significant age differences among the three farming groups. Moreover, there was no evident relationship between participant age and AChE activity depression. Even though hematological parameters might be affected by age and sex, the present study results showed that the selected hematological parameters and sex parameters had no effect and were all normal.

AChE activity was not significantly different for sex, occupation, pesticide used in rice planting, self-pesticide spraying, and last time pesticide used in rice planting. The average AChE activity fell within a normal range (Table 2).

However, 10 samples (11.49%) revealed borderline depressed AChE activity (ΔpH 1.0–1.15), of which half (five samples) were pesticide-using farmers. The AChE

activities of pesticide-exposed farmers were not critically depressed, but they were the largest group showing AChE activity depression (Table 3). The hematological effect of pesticides in this study was minor (Table 4). However, results indicated lower platelet numbers for self-spraying farmers who were directly exposed to pesticides than for those farmers who hired people to spray pesticides (Table 5).

Qualitative screening of samples from both non-pesticide and pesticide-using rice fields determined the pesticide residue that inhibited AChE activity (Table 6). Positive results from the screening assays indicated contamination of organophosphate and/or carbamate. The samples in this study were soil, water, and unmilled rice from each field. A total of 27 samples were collected from non-pesticide-using fields, and 28 samples were collected from pesticide-using rice fields. A total of 20 samples (74.07%) from non-pesticide fields tested positive for pesticide screening; however, this was less than the pesticide-using fields, which revealed 25 samples (89.29%) as positive. Five samples collected from pesticide-using fields tested positive at unsafe levels, whereas only three samples (11.11%) from non-pesticide fields tested at unsafe levels.

Table 1. Demographic Data

Variable	Category	n (%)	Occupation			p-Value*
			Pesticide-using Rice Farmers	Non-pesticide-using Rice Farmers	Non-Farmers	
Sex	Male	49 (48.00)	22 (59.46)	16 (51.62)	12 (35.29)	-
	Female	53 (52.00)	15 (40.54)	15 (48.38)	22 (64.71)	-
Age (year)	<40	24 (23.50)	8 (21.62)	8 (25.81)	8 (25.53)	-
	40–50	12 (11.80)	6 (16.22)	2 (6.45)	4 (11.73)	-
	51–60	44 (43.10)	17 (45.94)	15 (48.38)	12 (35.29)	-
	>60	22 (21.60)	6 (16.22)	6 (19.36)	10 (29.45)	-
Average age (year)		53.50	52.81	53.19	54.53	0.816
Total		102 (100)	37 (36.27)	31 (30.39)	34 (33.34)	

Note: *Mean comparison of age between pesticide-using rice farmers, non-pesticide-using rice farmers, and non-farmers using ANOVA

Table 2. Red Blood Cell Acetylcholinesterase Activity

Study Parameter	Category	N	AChE activity* $\Delta pH \pm SD$	p-Value**
Total of Sample	n	87	1.28 $\pm$ 0.10	
Sex	Male	46	1.29 $\pm$ 0.11	0.411
	Female	41	1.27 $\pm$ 0.09	0.411
Occupation	Farmer	60	1.29 $\pm$ 0.10	0.915
	Non-farmer	27	1.27 $\pm$ 0.10	0.915
Pesticide used in rice planting	Yes	34	1.28 $\pm$ 0.11	0.816
	No	26	1.30 $\pm$ 0.90	0.816
Self-pesticide spraying	No (hire)	19	1.28 $\pm$ 0.11	0.267
	Yes	15	1.29 $\pm$ 0.12	0.267
Last time that pesticide was used in rice planting	≤ 3 months	16	1.28 $\pm$ 0.12	0.940
	>3 months	17	1.29 $\pm$ 0.11	0.940

Notes: *Normal AChE activity: $\Delta pH > 1.15$, Borderline depressed AChE activity: ΔpH 1.0–1.15, Severely depressed AChE activity: $\Delta pH < 1.0$

**Independent t-tests

Table 3. Acetylcholinesterase Activity of Sample Groups

Sample group	n (%)	AChE activity*	
		Normal n(%)	Borderline depressed n(%)
Pesticide-using rice farmers	34 (39.08)	29 (85.29)	5 (14.71)
Non-pesticide-using rice farmers	26 (29.89)	24 (92.31)	2 (7.69)
Non-farmers	27 (31.03)	24 (88.89)	3 (11.11)
Total	87 (100.00)	77 (88.51)	10 (11.49)

Note: *Normal AChE activity: $\Delta pH > 1.15$, Borderline depressed AChE activity: $\Delta pH 1.0-1.15$, Severely depressed AChE activity: $\Delta pH < 1.0$

Table 4. Hematological Parameters

Parameters	Normal range	Mean $\pm$ S.D.			p-Value*
		Total sample n = 90	Farmers n = 61	Non-farmers n = 29	
Hb (gm%)	12.0–16.4	13.22 $\pm$ 1.73	13.38 $\pm$ 1.64	12.88 $\pm$ 1.87	0.194
Hct (%)	36.1–50.3	40.78 $\pm$ 5.09	41.19 $\pm$ 4.91	39.91 $\pm$ 5.44	0.267
WBC count ($\times 10^3$ cells/ μ l)	4.0–11.0	6.86 $\pm$ 3.46	6.79 $\pm$ 2.51	7.00 $\pm$ 5.43	0.778
WBC differentiation (%)					
Neutrophil	54–62	45.10 $\pm$ 11.18	46.26 $\pm$ 10.29	42.66 $\pm$ 12.69	0.154
Lymphocyte	25–35	42.49 $\pm$ 9.19	41.52 $\pm$ 9.11	44.52 $\pm$ 9.17	0.150
Monocyte	3–7	7.62 $\pm$ 2.39	7.75 $\pm$ 2.69	7.34 $\pm$ 1.56	0.367
Eosinophil	1–3	4.66 $\pm$ 4.50	4.31 $\pm$ 3.64	5.38 $\pm$ 5.92	0.295
Basophil	0–5	0.13 $\pm$ 0.40	0.15 $\pm$ 0.44	0.10 $\pm$ 0.31	0.630
Platelet (10^3 cells/ μ l)	140–400	274 $\pm$ 79	265 $\pm$ 84	291 $\pm$ 68	0.152

Notes: *p-value calculated by independent t-test between farmer and non-farmer groups; Hb = Hemoglobin; Hc = Hematocrit; WBC = White blood cells

Discussion

According to the participating farmers' demographic data, the majority of samples were males between 51 and 60 years old. This result was consistent with the general characteristics of a Thai farmer; that is, male and older. A documentary by the Food and Agriculture Organization (2000) stated that only the older generation continues to farm rice. It was also noted that the average age of the farmers in the study area was higher than the average age of those in Thailand (46.01 years old).¹⁵ This result is also consistent with socio-economic study of rice farmers, which found that most farmers were male.¹⁶⁻¹⁸

In this study, RBC AChE was selected for determination instead of serum cholinesterase (ChE). This is because AChE is the true cholinesterase that is present in the brain and erythrocyte, while ChE is pseudo cholinesterase that is present only in serum. Prior studies demonstrated that AChE activity was suppressed by every organophosphate used in the experiment, including fenthion, chlorpyrifos, diazinon, bromophos, propaphos, haloxon, and DFP, while only a few organophosphates can be suppressed in serum isoenzyme ChE.¹⁹

The average AChE of all samples was normal. However, approximately 11.49% (10 samples) had borderline depressed AChE activity. Among this group, half were pesticide-using farmers. These results indicate that pesticide-using farmers were at high-risk of AChE inhibi-

tor exposure, confirming several previous results.²⁰⁻²³ The other five depressed AChE activity samples consisted of two non-pesticide-using rice farmers and three non-farmers. This result indicates that they might have been exposed to pesticides, especially AChE inhibitors that contaminated food, water, and the environment, possibly from home pesticide products.^{24,25}

As seen in Table 4, the blood indices for farmers and non-farmers were not significantly different. However, if analyzing the difference self-pesticide spraying and non-self-spraying farmers, the results showed a difference in platelet count that was lower in the self-spraying group. These findings indicated that self-spraying pesticide farmers had a higher chance of exposure to pesticides, which adversely affected their platelet numbers. Previous reports showed that pesticide exposure resulted in hematological changes not only in platelets but also white and red blood cells.²⁵ AChE is found in neuron synapses and red blood cells, as well as in platelets and megakaryocyte cells.²⁵ Hence, the inhibition of AChE might affect platelet numbers. However, because the participant ages averaged 53.50 years, it is possible that old age might be the influencing factor for the effect seen in the hematological indices.^{26,27}

In this study, pesticide and non-pesticide-using farmer groups did not show significantly affected hemoglobin and hematocrit levels compared to the control and non-

Table 5. Hematological Parameters of Rice Farmers Using Pesticides

Parameter	Mean $\pm$ S.D.		p-Value*
	Self-pesticide Spraying n = 16	Non-self-pesticide Spraying n = 16	
Hb (gm%)	13.59 $\pm$ 1.24	13.38 $\pm$ 1.06	0.588
Hct (%)	41.79 $\pm$ 3.43	40.96 $\pm$ 3.81	0.513
WBC count (103 cells/ μ l)	6.92 $\pm$ 2.32	6.83 $\pm$ 2.89	0.910
Neutrophil	45.31 $\pm$ 9.50	46.67 $\pm$ 11.31	0.710
Lymphocyte	42.25 $\pm$ 8.68	39.89 $\pm$ 8.04	0.416
Monocyte	8.44 $\pm$ 3.29	7.56 $\pm$ 3.15	0.430
Eosinophil	4.00 $\pm$ 2.13	5.61 $\pm$ 5.40	0.255
Basophil	0.00 $\pm$ 0.00	0.28 $\pm$ 0.57	0.056
Platelet (103 cells/ μ l)	214 $\pm$ 67	283 $\pm$ 77	0.010**

Notes: *p-value calculated by independent t-test, **: significant different (p-value < 0.05);
Hb = Hemoglobin; Hc = Hematocrit; WBC = White blood cells

Table 6. Qualitative Pesticide Residue Contamination in Rice Samples and the Environment

Sample type	Pesticide Residue Contamination ^a							
	Samples of Non-pesticide Fields (%)				Samples of Pesticide Fields (%)			
	No.	Negative ^b	Positive		No.	Negative ^b	Positive	
			Safety Level ^c	Unsafety Level ^d			Safety Level ^c	Unsafety Level ^d
Soil	11	4 (36.36)	4 (36.36)	3 (27.28)	13	1 (7.69)	8 (61.53)	4 (30.78)
Water	8	-	8 (100.00)	-	11	1 (9.09)	9 (81.82)	1 (9.09)
Unmilled rice	8	3 (37.50)	5 (62.50)	-	4	1 (25.00)	3 (75.00)	-
Total	27	7 (25.93)	17 (62.96)	3 (11.11)	28	3 (10.71)	20 (71.43)	5 (17.86)

Notes: ^a Pesticide residue determined by GT-Pesticide Residual test kit, ^b Negative: pesticide level lower than 0.05 mg/kg (detection limit), ^c Positive with safe level: pesticide detected but within safety level, ^d Positive at unsafe level: pesticide detected at an unsafe level

mal range levels. However, surveillance and caution must be maintained in the use of pesticides. Contaminated environmental and rice samples from pesticide-using fields screened positive at a higher rate than non-pesticide using fields. Results confirm that farmers in pesticide using fields had higher organophosphate and/or carbamate exposure risk from pesticide self-spraying and rice consumption. The 74.04% positive samples from non-pesticide fields might have been contaminated from nearby fields. Therefore, non-pesticide-using rice fields in our study were non-true pesticide residue-free areas. Findings indicate that pesticide-free planting still experienced management problems within the study area.

Conclusion

Half (50%) of AChE borderline depressed activity sample participants are pesticide-using farmers, while the others are both pesticide-using farmers and non-farmers. Most hematological parameters are normal in all samples. However, platelet numbers of pesticide self-spraying rice farmers are significantly lower than non-self-spraying rice farmers. The results suggest that the use of pesticides in rice farming affects AChE activity and some hematological indices. Besides, pesticide residue environment samples, and rice from pesticide fields show higher pesticide contamination rates than in non-pesticide areas. These results indicate and confirm that the farmers in

pesticide-using areas are at a higher risk of pesticide exposure than non-pesticide-using farmers.

However, this study was conducted in only one sub-district of Nakhon Si Thammarat Province. Therefore, further study is recommended in a wider area that covers all provinces of Southern Thailand. Moreover, the further study might include additional agricultural farmer types other than just rice farmers, such as oil palm planters and rubber planters; these are both famous occupations in Southern Thailand and may also be interesting to study.

Acknowledgment

This study was supported by a grant from the Area-based Collaborative Research Project between the Thailand Research Fund and Walailak University. The contributions of each author are as follows: WK was the main operator and corresponding author. SY was the chief operator and co-author. SJ, PT, JM, and UV were the co-authors.

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