

Biochemical and Hematological Status in Rice farmers with Chronic Pesticide Exposure, Suphan Buri, Thailand

Kowit Suwannahong¹, Airada Sridon², Wanida Pongstaporn³, Pimporn Thongmuang⁴, Yuttana Sudjaroen⁵

¹Assistant Professor, Department of Environmental Health, Faculty of Public Health, Burapha University, Thailand, ²Research scholar, Department of Forensic Science, Faculty of Allied Health Sciences, Thammasat University, Thailand, ³Assistant Professor, Department of Medical Science, Faculty of Science, Rangsit University, Thailand, ⁴Assistant Professor, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand, ⁵Assistant Professor, Department of Applied Sciences, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Thailand

Abstract

Suphan Buri is located in central river plain of Thailand, which has enough water supplies for rice cultivation area in two or three growing cycles. Thus, farmers may expose to pesticides more frequent rather than other area and may become as chronic pesticide exposure. Aim of this study was to determine biochemical and hematological of rice farmers compared with controls who living nearby agricultural area. Subjects were divided to rice farmers (N = 50) and controls (N = 50). Individual data, pesticide-exposure status and relating symptoms were documented before blood collection. Serum samples were analyzed for serum cholinesterase (SChE) activity, liver function test (LFT) and kidney function test (KFT). Each EDTA-whole blood was determined for complete blood count (CBC). SChE activity was screened and confirmed by paper test and automatic analyzer, respectively. LFT, KFT and CBC were run by automatic analyzers. Results were represented in descriptive data and difference of LFT and hematological status between two groups were evaluated by independent *t*-test ($p < 0.05$). 60% of rice farmers were used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. Means of SChE level of both groups were within reference value and significantly different ($p = 0.033$). Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) values of rice farmers were significantly lower ($p = 0.045$, 0.04 and 0.03, respectively). SChE activity and biochemical parameters in rice farmers were within reference value; therefore disturbance of hematological status, such as anemia was appeared.

Keywords: blood indices, chronic pesticide exposure, kidney function test, liver function test, serum cholinesterase, complete blood count

Introduction

Pesticides are broadly utilized for pest and pest-induced disease controls, especially in crop cultivation

and vector-borne diseases control for public health work [1]. In developing countries were used pesticide about 20% of the world and Thailand was the third rank of pesticide usages in Asia-Pacific region [2]. The reported cases of the toxic effects of pesticide are predominantly in the Central region of Thailand. The numbers of cases were usually increased during the growing season of many crops in rainy season (May - August) each year, and it was found mainly in farmers and farm workers [3]. Central of Thailand is on intensively cultivated rice crop land when water conditions allow had been occurred [4]. Suphan Buri province is located in central plain

Corresponding Author:

Yuttana Sudjaroen

Forensic Science Program, Department of Applied Sciences, Suan Sunandha Rajabhat University, U-Thong Nok road, Dusit, 10300 Bangkok, Thailand
Contact: +66-2-1601143; Fax: +66-2-1601146;
E-mail: yuttana.su@ssru.ac.th

and has enough water supplies in this cultivation area, two or three rice growing cycles. Thus, farmers may expose to pesticides more frequent rather than other area also. Agricultural workers in Suphan Buri had reported using several brands of insecticides, plant hormones, and chemicals for “control of plant diseases”. Most of active insecticide substances in commercial products are abamectin, chlorpyrifos, carbofuran, and cypermethrin [3, 5].

Various effects of chronic pesticide exposure are cancer, birth defects, reproductive disorders, neurodegenerative, cardiovascular and respiratory diseases, developmental disorders, metabolic disorders, chronic renal disorders or autoimmune diseases [6]. According by epidemiologic data, pesticides are effect on enzymes, which are responsible for liver function, blood cell characteristics and other biochemical pathways in persons who are pesticide expose and have occupationally diseases [7-9]. Biological effects of pesticide are involved by oxidative stress, epigenetic controls and gut microbiological digestion and modulation of genetic polymorphisms [10, 11]. Light occupational exposure to pesticides is associated with hematological abnormalities [12]. However, prolong and intensive exposure may associate to any liver, kidney, or hematological disorders. This study was aimed to determine biochemical and hematological parameters in rice farmers, which were intensively pesticide exposed and habituated in rice cultivation area of Suphan Buri, Thailand. The finding may indicate the association between pesticide exposure and early subtle and sub-clinical changes in biochemical and hematological parameters.

Materials and Methods

Subject recruitment and data collection

The study was conducted in U-Thong district, Suphan Buri province, about 150 km from West of Bangkok, where rice is main harvesting product. Outsource pesticide sprayers were common finding in rice field. Cross-sectional study was carried out from June 2019 to February 2020 on data had collected from annually health service program by health promoting hospital. This study was recruited 100 respondents included 1) 50 rice farmers (risk group) were aged 18-65 yr who lived in this area, which had handle pesticide regularly

or work in paddy field at least three years or more 2) The control group was included 50 respondents who lived nearby field area and listed in house registration, had non-related professional for farm workers. Respondents with a history of serious conditions, such as, liver diseases, severe cardiovascular diseases, cancer were excluded. Questionnaire interviewing and blood collection were conducted by well-trained research assistants and medical technologists, respectively. Gathered information concerning of long-term pesticide exposure was recorded from questionnaires by personnel interviewing. The sample size was estimated using the single proportion formula with 95% confidence interval and based on percentage of abnormal SChE level in previous study [13]. The Ethics Committee of Thammasat University was approved this research protocol (COA No. 084/2562). The director of U-Thong district & health promoting hospital, Suphan Buri province gave permission to conduct on this study. All participants gave informed consented.

Blood collection, preparation and storage

Each 5 ml of blood sample was obtained by venipuncture from median cubital vein during morning (7-9 a.m.); and drawn into clotting blood and EDTA tubes for 3 ml and 2 ml, respectively. Clotting blood tube was further centrifuged; and serum was separated within 2 h after phlebotomy and stored at -20 °C [14] for SChE activity, LFT and KFT analysis. Whole blood contained in EDTA tubes were prepared for CBC test.

Evaluation of biochemical and hematological parameters

SChE screening and confirming of SChE level was done by paper test and automatic analyzer, respectively. The paper test kit was developed and manufactured by Government Pharmaceutical Organization (GPO), Thailand. The efficiency of test including sensitivity, specificity and positive predictive values were 77, 90 and 85%, respectively [15]. The quantitative analysis of SChE activity, LFT and KFT was conducted by automatic analyzer, COBAS c501 (Roche-diagnostics, Rotkreuz, Switzerland), which were performed in certified clinical laboratories. All hematologic parameters in CBC were analyzed by Celltac E MEK-7222 (Nihon Kohden, Tomioka, Japan). Interpretation of biochemical and hematological parameters was done by reference value

comparing according by instruction of manufacturer and the Clinical and Laboratory Standards Institute (CLSI).

Statistical Analysis

Descriptive data was explained by using mean and standard deviation; and frequency. The Kolmogorov-Smirnov test was used to test for normal distribution of data. Independent *t*-test tested for differences in the biochemical and hematological parameters between rice farmers and controls. The statistical significance was judged at $p < 0.05$. SPSS 21.0 software was used for statistical analysis (SPSS, Chicago, Illinois, USA).

Results and Discussion

Chronic pesticide exposure in rice farmers

No statistical significance different between rice farmers and controls for gender and risked behavior, such as alcoholic consumption. All of rice farmers were long-term pesticide exposure and 60% of rice farmers were used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. The related pesticide used symptoms were rarely occurred and almost of them had health education for awareness of pesticide uses. However, unexpected finding may due to unspecific symptoms, imprecisely explain by personal interviewing and tolerance of frequent exposed farmers. Means of SChE level were significantly different between rice farmers and controls ($p = 0.033$), however, there were within reference value (5,500-13,000 U/L). Some of rice farmers had SChE level lower than reference value (data not show), which was implied that SChE may not be a good marker for quantifying exposure to pesticide among sprayers, especially during spraying season. In previous study, agriculturists can be exposed to pesticides divided into sprayers, agriculturists and other professions, however, the SChE levels among them were not significantly difference and level was still within reference range^[16]. Most of studies on Thai pesticide exposure had reported exposure of

single type of pesticide; however, mixed pesticide uses are more common for multi-crop cultivation. Thus, screening of other biomarkers for evaluation of long-term pesticide exposure rather than serum cholinesterase such as, alkyl phosphate metabolites (DAPs), urinary 3-phenoxybenzoic acid (3-PBA) and urinary glyphosate, is still necessary for public provider^[17].

Biochemical and hematological parameters in rice farmers

Biochemical parameters were not significantly different between rice farmers and controls; and there were within reference values (Table 1). Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) values of rice farmers were significantly lower than controls ($p = 0.045$, 0.04 and 0.03, respectively); and MCHC in rice farmers was lower than reference value (Table 2). Subclinical change in hematological parameter is little attention on pesticide exposure rather than cancer, neurological disorders, epigenetic interaction, oxidative stress and gene polymorphism^[10-12]. However, hematotoxicity is biomarker and can be indicate occupational exposure along with genotoxicity and oxidative stress occurrence^[18]. In this study, the mean of MCHC was lower in pesticide sprayers than reference range. Low MCHC was represented mild anemia may have been caused by 1) anemia in some elderly farmers arising from a condition such as chronic blood loss and/or malnutrition and 2) the sub-chronic pesticide exposure especially OPs may induced anemia in animal models^[19, 20]. In previous studies, abnormal blood indices had finding and the impairment of liver and kidney functions had apparent in pesticide sprayers and agricultural workers^[21-24]. Lifetime and high season pesticide use are also associated with lower number of RBC and WBC, particularly lymphocyte and eosinophil^[25].

Table 1: Liver and kidney function tests between rice farmers and controls

Group/ parameter	Liver function test							Kidney function test	
	Total protein (g/dL)	Albumin (g/dL)	Total bilirulin (mg/dL)	Direct bilirulin (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Rice farmer	7.29 ± 0.22	4.50 ± 0.04	0.36 ± 0.02	0.13 ± 0.03	23.0 ± 1.2	21.7 ± 2.0	99.9 ± 3.5	10.8 ± 4.6	0.85 ± 0.1
Control	7.45 ± 0.16	4.43 ± 0.04	0.36 ± 0.04	0.11 ± 0.01	23.6 ± 1.3	24.6 ± 1.9	101.2 ± 4.0	9.3 ± 3.5	0.71 ± 0.1
Reference range	6.60-8.70	3.50-5.50	0.30-1.20	0.00-0.50	0-37.0	0-40.0	53-128	5.0-23.0	0.5-1.52
p-value	0.552	0.282	0.964	0.485	0.744	0.308	0.797	0.561	0.152

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, BUN = blood urea nitrogen

Table 2: Hematological parameters in rice farmers and controls *

Group/ parameter	RBC (10 ⁶ /mm ³)	Hct (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	WBC (10 ³ /mm ³)	Plt (10 ³ /mm ³)
Rice farmer	5.16 ± 0.1	41.8 ± 0.8	13.5 ± 0.3	83.4 ± 1.3	26.0 ± 0.7	30.3 ± 0.2	15.3 ± 0.6	5,895 ± 265	264,421 ± 9,627
Control	5.13 ± 0.1	41.8 ± 1.2	14.5 ± 0.6	88.4 ± 1.6	27.8 ± 0.6	31.7 ± 0.7	15.0 ± 0.5	5,699 ± 241	247,802 ± 10,424
Reference range	4.0-5.5	M = 35-49 F = 32-42	M=13-18 F=12-16	80-100	23-33	31-37	12-16	4,000-10,000	140,000-400,000
p-value	0.84	0.984	0.154	0.045	0.04	0.03	0.757	0.587	0.244

WBC = white blood cell, RBC = red blood cell count, Hct = hematocrit, Hb = hemoglobin, MCV = mean corpuscular volume,

MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = RBC distribution width,

Plt = platelet count

* RBC morphology and WBC differentiation with microscopic examination were not shown

Conclusion

Rice farmers with frequent and prolonged exposure in this study area were normal SChE and biochemical parameters; therefore disturbance of hematological status, such as anemia was appeared. Blood indices may use as biomarkers to evaluate early subtle and sub-clinical changes in chronic pesticide exposure.

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Ethical Clearance: Ethics Committee of Thammasat University was approved this research protocol.

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

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