Rocz Panstw Zakl Hig 2015;66(1):21-26

AN ASSOCIATION BETWEEN ORGANOPHOSPHATE PESTICIDES EXPOSURE AND PARKINSONISM AMONGST PEOPLE IN AN AGRICULTURAL AREA IN UBON RATCHATHANI PROVINCE, THAILAND

Saowanee Norkaew[1], Somrat Lertmaharit[1, 3], Wachiraporn Wilaiwan[1], Wattasit Siriwong[1, 2], Héctor Maldonado Pérez[4] and Mark G. Robson[2, 4]*

[1] College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand
[2] Thai Fogarty ITREOH Center, Chulalongkorn University, Bangkok, Thailand
[3] Department of Preventive & Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[4] School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA

ABSTRACT

Background. *Parkinson's* disease (PD) is a ubiquitous disease. However, PDs prevalence in the population of agricultural communities lacks understanding and there has been no epidemiological study on the association between pesticides exposure factors and risk for PD.

Objective. To investigate the potential association between organophosphate pesticides exposure and Parkinsonism by using a screening questionnaire in agricultural areas.

Material and Methods. Ninety elderly people living in agricultural areas participated in a cross-sectional study conducted at Tambon Hua-Rua Health Promoting Hospital in April 2014. Screening questionnaires for *Parkinson's* disease, Test-mate ChE (Model 400) for blood cholinesterase (ChE) levels of both blood enzymes erythrocyte cholinesterase (AChE), and plasma cholinesterase (PChE) were used as measurement tools. Descriptive statistics for frequencies and percentage distributions were used primarily to summarize and describe the data. Sensitivity, specificity, positive and negative predictive values were calculated.

Results. The age range of the participants was 50 to 59 years old, with an average age of 53.9±2.87 years. The majority of the participants were female (62.2%), 82.2% of respondents were farmers. Most of participants (76.7%) reported that they applied insecticides in their farms. Ninety persons participated and completed the 11-item questionnaire. Of these, 17 (18.9%) felt that they lost balance when turning or that they needed to take a few steps to turn right around and 16.7% of participants indicated that they felt the need to move slowly or stiffly. The study found the prevalence of abnormal AChE levels was 28.9% (95%CI=19.81-39.40) and 17.8% of PChE levels (95%CI=10.52-27.26). To predict Parkinsonism, AChE, and PChE level, with a cutoff score of 5 or higher there had to be a sensitivity of 0.31, specificity of 1.00, positive predictive value (PPV) of 1.00 and negative predictive value (NPV) of 0.78 for AChE. While PChE, the score value of 5 or more had a sensitivity of 0.19, specificity of 0.93, PPV of 0.38 and NPV of 0.84.

Conclusion. This study described an association between pesticides exposure and Parkinsonism. The questionnaire appears to be useful for Thai agriculturists as a screening tool for Parkinsonism and cholinesterase levels regarding to pesticides exposure.

Keywords: *organophosphate, pesticides exposure, Parkinsonism, cholinesterase activity*

INTRODUCTION

Parkinson's disease (PD) is a degenerative disorder, a type of movement disorder. It happens when dopamine, a brain chemical is not produced enough by nerve cells in the brain. One's genetic makeup is believed to be a cause of PD; chemical exposure in the environment might play a role, but most cases do not seem to run in families [1]. Currently, there are many types of pesticide exposures that may occur through contaminated water and food [2]. Some studies have linked the geographical distribution of pesticides usage with the prevalence of PD [3-6]. The symptoms of PD may present as one or more of the following categories: primary motor symptoms, secondary motor symptoms, non-motor symptoms, and coping with symptoms. Primary motor

***Corresponding author:** Wattasit Siriwong, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand, e-mail: Wattasit.S@chula.ac.th

symptoms include resting tremors, slowness of movement, and poor balances are examples [7]. Currently, it is difficult to diagnose PD. There is no laboratory test for PD that can be administered for diagnosis; this is why doctors use a patient's medical history and a neurological examination to make the diagnosis. PD is common in the elderly and begins developing around the age 60, but it can start earlier. Some of the previous studies reported the average ages of PD patients were 55 to 60 year-olds and most of the patients were more than 50 years of age [3, 8, 9]. It is more often found in men than in women. Currently, there is no cure for PD but early diagnosis and medical care can help slow down the progression of the symptoms and help the patient have a better quality of life [1].

At present, Thailand has no study on the statistics of PD for prevalence or incidence rate. Thai *Parkinson's* disease registry project was started in March 2008. There are currently more than 60,000 patient cases registered. The prevalence of PD in Thailand was 424.57 PD cases per 100,000 persons. The largest prevalence of PD was in the Central Region of Thailand, higher than the rate of the overall population in the country. This may suggest that the variation is caused by the different amounts of chemicals in use but never confirmed by a check-up and/or drawing patient blood samples, consequently, it was only a presumption [9]. From the previous studies in PD patients it was shown that patients who were exposed to pesticides, organochlorines and paraquat correlated to having PD [10, 11] and occupational exposures to pesticides were linked with PD [12].

Agricultural work is an important sector that can strengthen Thailand's economy. Some of the problems these workers encounter are health related problems caused by toxic chemicals such as pesticides that are used for pest control and agricultural application. As a result, agriculturists as well as the general public may be exposed to such substances through ingestion, inhalation, and dermal absorption [13]. One biomarker that can be used to measure the effect of pesticides is the activity of cholinesterase enzymes [14]. An annual report of the Bureau of Occupational and Environmental Diseases has concluded that the cholinesterase enzymes in blood of agriculturists across Thailand had depressed levels. In 2002, out of 563,354 persons it was founds that 89,926 persons (15.96%) had risky and unsafe cholinesterase levels [15].

Ubonratchathani province is a province in the Northeastern of Thailand. It is the agricultural area that mainly grows chili. The main districts that grow chili are in the province of the Mueang district, Muang Sam Sip district, Khueang Nai district, Sirindhorn district, and Phibun Mangsahan district. The province has a growing chili area is 6,605 Rais (1 rai = 0.4 acre), where 11,229 tons of chili product are produced per year. Chili farming activity occurs from December until April, chili farmers also grow rice after they harvest the chili. Chili farmers apply fairly high rates of pesticides [16], the province had 42 patients poisoned by pesticides or 2.35 per hundred thousand persons in the populations with no mortality in 2008. In 2009 reports, the province had 75 patients poisoned by pesticides or 4.20 per hundred thousand, there are no mortality statistics. In 2009 cholinesterase enzyme levels in 3,321 agriculturists were studied and it was found that 1,053 agriculturists, that is 31.71% of the agriculturist, had depressed cholinesterase levels. As a result, agriculturists in Ubon Ratchathani who grew vegetables such as chili or fruits by using pesticides had risky and unsafe cholinesterase enzyme levels higher than the average risky and unsafe cholinesterase enzyme levels in agriculturists across the country (annual report in Hua-Rua Health Promoting Hospital, 2011). The pesticides that chili farmers in the Hua-Rua sub-district, Mueang district, Ubon Ratchathani Province used were organophosphate pesticides (OP) such as chlorpyrifos and profenofos [16,17].

There is no data on the prevalence of PD in the agricultural area in the Hua-Rua sub-district, Mueang district, Ubonratchathani Province and there was no information on the association between pesticides exposure factors and the risk for PD. Our research aim is to investigate the possible association between pesticides exposure and PD by using a screening questionnaire among elderly people living in chili farming community who may be at risk group because of senility, employment in an agricultural occupation, and continued pesticides exposure including the use of pesticides in household and residential areas.

MATERIAL AND METHODS

This study was cross-sectional study. The study population was primarily elderly persons who were living in the Hua-Rua agricultural community. There are 90 elderly people living in the study area. Previous studies have shown the average age range of PD patients is 55 to 60 years old [3, 8, 9]. The participants were selected by purposive sampling; age between 50 to 59 years, included men and women. Those who have a history of cardiac disease, renal or hepatic insufficiency diagnosed by a doctor, using any neurological related medication and had Injuries/Head Trauma that had caused any type of harm to brain or central nervous system were excluded from the study. Representatives recruited participants from each house (one subject per household). Health care officers at the Tambon Hua-Rua Health Promoting Hospital performed the purposive sampling

with the criteria used to obtain the target sample number (90 participants).

Measurement tools

1. Questionnaire
   The questionnaire is separated into two parts: (1) to obtain general information and individual background and (2) a screening questionnaire for *Parkinson's* disease. The screening questionnaire for *Parkinson's* disease, originally come from *Setthawatcharawanich* et al. [18]. The questionnaire consisted of 11 questions with an answer of yes-no for the questions.

2. Blood test by Test-mate ChE (Model 400), EQM
   The Test-mate ChE Cholinesterase Test System is based on the Ellman method. Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or PChE, producing carboxylic acid and thiocholine, respectively, with reaction to the Ellman reagent (DTNB, dithionitrobenzoic acid) so as to create a yellow color that is gauged spectrophotometrically at 450 nm. The rate of color formation is in proportion to the amount of either AChE or PChE [19]. Nurses collected 20 μL of blood per person from the participants at Tambon Hua-Rua Health Promoting Hospital, in which optimum temperature was controlled at less than 30 °C as recommended by Test-mate ChE Cholinesterase Test System (Model 400) specification. The analysis of cholinesterase levels in erythrocyte and plasma using Test-mate was also performed.

Data analysis

Interpreted level of cholinesterase: for AChE, if values are less than or equal to 2.92 U/mL this indicates possible pesticide poisoning thus the participant should be removed from the exposure and/or treated with anticholinergics. A cholinesterase level of more than 2.92 U/mL value is considered normal. For PChE, if the values were less than or equal to 1.56 U/mL it indicates possible pesticide poisoning and the participant should be removed from the exposure and/or treated with anticholinergics. A cholinesterase level of more than 1.56 U/ mL value is considered normal (U/mL reference from Test--mate ChE (Model 400), EQM).

Using a licensed SPSS Version 16 for windows, general characteristics and socio-demographic were described by frequency, percentage, and mean. Sensitivity, specificity, a positive predictive value (PPV) and a negative predictive value (NPV) were calculated. PPV; was defined as the number of true-positive studies divided by the sum of true-positive and false-positive studies and NPV; was defined as the number of true--negative studies divided by the sum of true-negative and false-negative studies.

Ethics consideration

The experimental protocol was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Sciences Group I, Chulalongkorn University with the certified code no.056/2014.

RESULTS

General characteristics and socio-demographic

The participants ranged from 50 to 59 years old, with an average age of 53.9±2.87 years. The majority of the participants were female (62.2%) and 82.2% of the respondents were farmers. Most of participants (76.7%) indicated that they applied insecticides on their farms. 61.1% of respondents reported that the source of drinking water for their family was the tap water. In the past six months, approximately 58.9% of respondents suffered some type of illness. Table 1 describes sample population profile.

Table 1. Socio-demographic characteristics of the respondents (n=90)

Characteristics	Number (n=90)	Percentage (%)
Gender		
Male	34	37.8
Female	56	62.2
Occupation		
Unemployed	4	4.50
Chili farmers	37	41.1
Rice farmers	37	41.1
Others	12	13.3
Insecticide uses		
Yes	69	76.7
No	21	23.3
Source of drinking water		
Tap water	55	61.1
Ground water	17	18.9
Well water	2	2.20
Others	16	17.8
Frequently of illness in the past six months		
Never	36	40.0
Sometimes	53	58.9
Usually	1	1.10

A screening questionnaire for Parkinson's disease

Ninety persons participated and completed the 11-item questionnaire. Of those who completed the survey, 17 (18.9%) felt that they lost balance when turning or that they needed to take a few steps to turn right around, 16.7% of participants indicated that need to move slowly or stiffly and 15.5% of respondents felt that it was difficult to get up again after sitting down. Table 2 demonstrates the number of participants giving a positive answer to each question on a screening questionnaire for *Parkinson's* disease.

Table 2. Number of participants giving a positive answer to each question on a screening questionnaire for *Parkinson's* disease (n=90)

Items	n	%
1. Have you noticed that you become clumsier or have more difficulty with tasks that involve fine hand control: for example, doing up your buttons?	5	5.60
2. Have your handwriting changed and become smaller compared to when you were young?	0	0
3. Do you feel you move slowly or stiffly?	15	16.7
4. Do you walk with a stooped posture?	11	12.2
5. Have you noticed that you do not swing your arms when you walk as much as you used to?	6	6.70
6. Do you find it difficult to start walking from a standstill or have difficulty in stopping suddenly when you want to?	5	5.60
7. Have you noticed that a tremor of your hands, arms, legs or head?	7	7.80
8. Do you have a lack of facial expression or tend to drool with your mouth half-open?	5	5.60
9. Have you noticed that your voice has become softer or more monotonous?	8	8.90
10. When you turn, do you lose balance or do you need to take quite a few steps to turn right around?	17	18.9
11. After you sit down, do you find it difficult to get up again?	14	15.6

Prevalence of abnormal ChE levels both AChE and PChE

The study found that the prevalence of abnormal AChE levels was 28.9% (95%CI=19.81-39.40) and 17.8% of PChE levels (95%CI=10.52-27.26). Table 3 demonstrates the number and percentage of participants with cholinesterase activities.

Table 3. Number and percentage of participants with cholinesterase activities resulted

	n	%	95% CI
AChE			19.81-39.40
Abnormal*	26	28.9	
Normal	64	71.1	
PChE			10.52-27.26
Abnormal**	16	17.8	
Normal	74	82.2	

* Less than or equal 2.92 U/mL
** Less than or equal 1.56 U/mL

Performance characteristics of AChE, PChE and a screening questionnaire for Parkinson's disease

Sensitivity, specificity, a positive predictive value and a negative predictive value were calculated for sum of the simplified score using all 11 questions with AChE and PChE level. To predict Parkinsonism and AChE and PChE level, the score value of 5 or more had a sensitivity of 0.31, specificity of 1.00, PPV of 1.00 and NPV of 0.78 for AChE. While PChE, the score value of 5 or more had a sensitivity of 0.19, specificity of 0.93, PPV of 0.38 and NPV of 0.84 (Table 4).

Table 4. Performance characteristics of AChE, PChE and a screening questionnaire for *Parkinson's* disease at different cut-off points

	Cut-off	Sensitivity	Specificity	PPV	NPV
AChE	≥3	0.50	0.98	0.93	0.83
	≥4	0.50	0.98	0.93	0.83
	≥5	0.31	1.00	1.00	0.78
	≥6	0.08	1.00	1.00	0.73
PChE	≥3	0.25	0.86	0.29	0.84
	≥4	0.25	0.86	0.29	0.84
	≥5	0.19	0.93	0.38	0.84
	≥6	0.13	1.00	1.00	0.84

Abbreviations:
PPV; positive predictive value, NPV; negative predictive value

DISCUSSION

In this study, it was found that the average AChE of participants was 3.31 ±0.56 U/mL. The AChE activity in this study was higher than the AChE activity in the previous study, which showed that AChE activity in farm workers was (2.63 ±0.55 U/mL) and in non-farmers was (2.80 ±0.53 U/mL) [20]. The possible reasons are both that growing different products and crops is associated with different exposure to organophosphates, and consequently with different AChE activity or that the previous study was conducted in high pesticides usage areas. The study showed that farmers are likely to have lower AChE activity than non-farmers as stated in the Argentina [21] study that compared AChE between direct and indirect exposed groups. The present study found the prevalence of abnormal AChE levels to be 28.9% and 17.8% for PChE levels. This study described that residential pesticides exposure among people who live in agricultural communities are possibly exposed to pesticides indirectly by their main occupation in the community.

A validated questionnaire for *Parkinson's* disease can be useful as a screening as well as a decrease time for the patient to complete the questionnaire. However, a physical examination of positive screening questionnaire should be concern for a final diagnosis. From the statistical analysis the respondents that felt to lose balance when turning or that needed to take a few steps before turn right around, felt that they moved slowly or stiffly and that 15.5% of respondents felt difficulty to get up again after sitting down. The results were similar to previous studies, which showed that motoric disorders are the main symptoms for Parkinsonism diagnosis [6, 22, 23]. Previous studies suggested that the presence of tremors was a good

indicator and recognizing characteristic [24, 25] as tremors are the most common presenting symptom of *Parkinson's* disease [26]. However it may not be a reliable predictor in a community group [27]. In 2000, *Chan* et al. reported the validation of screening questionnaires and found that 93.7% of *Parkinson's* disease patients in hospital group identified tremor while 62.5% in the community group.

This study indicated that the score value of 5 or more can be used as a cutoff score for predicting *Parkinson's* disease and pesticide exposure. These results were similar to previous studies which reported that a cutoff score of 5 or more ensured the best balance between sensitivity and specificity [18, 27].

CONCLUSIONS

Many previous studies have linked pesticides use with *Parkinson's* disease. In this study it was found that the study area uses a wide variety of pesticides. People in the community were exposed to pesticides used in their community and it could be assumed that people in this community may be exposed either by multiple pathways. From the results, it seemed likely that people's exposure to pesticides may be associated with an increased risk of *Parkinson's* disease or Parkinsonism as well. A screening questionnaire tested in this study is a useful tool to detect *Parkinson's* disease. However, the questionnaire should not replace the physical examination and the medical documentation of *Parkinson's* disease, which should be used to confirm the results of the questionnaire. In addition, the screening questionnaire can be used as a screening tool for Parkinsonism and pesticides exposure. The recommendation is to reduce pesticides exposure to reduce risk from *Parkinson's* disease. Risk management and risk communication is critical for the prevention and reduction of PD risk.

Acknowledgements

The authors thank Associate Professor Suwanna Setthawatcharawanich, M.D. for her permission to use the Thai version questionnaire and grateful to our participants for their encouragement of this study. This research was supported by the Rachadapisek Sompoth Fund Post-Doctoral Support, Chulalongkorn University, the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (AS581A-56) and the Ratchadapisek Sompoch Endowment Fund (2014), Chulalongkorn University (CU-57-066-AS), and NIH FIC under grant D43TW007849 and the NIEHS CEES ES020522.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Health NIo. Parkinson's disease 2014. Available from: http://www.nlm.nih.gov/medlineplus/parkinsonsdisease.html
2. *O'Malley M.:* Clinical evaluation of pesticide exposure and poisonings. The lancet. 1997; 349(9059):1161-6.
3. *Le Couteur D, McLean A, Taylor M, Woodham B, Board P.*: Pesticides and Parkinson's disease. Biomed Pharmacother 1999; 53(3):122-30.
4. *Engel L, Checkoway H, Keifer M, Seixas N, Longstreth W, Scott K, Hudnell K, Anger WK and Camicioli R.*: Parkinsonism and occupational exposure to pesticides. Occup Environ Med 2001; 58(9):582-9.
5. *Freire C, Koifman S.*: Pesticide exposure and Parkinson's disease: epidemiological evidence of association. Neurotoxicology 2012; 33(5):947-71.
6. *Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, Alpérovitch A and Tzourio C.*: Professional exposure to pesticides and Parkinson disease. Ann Neurol 2009; 66(4):494-504.
7. Foundation PsD. Symptoms [cited 2014]. Available from: http://www.pdf.org/symptoms
8. *de Lau LM, Breteler M.:* Epidemiology of Parkinson's disease. The Lancet Neurology. 2006; 5(6):525-35.
9. *Bhidayasiri R, Wannachai N, Limpabandhu S, Choeytim S, Suchonwanich Y, Tananyakul S, Tharathep C, Panjapiyakul P, Srismith R and Chimabutra K.:* A national registry to determine the distribution and prevalence of Parkinson's disease in Thailand: implications of urbanization and pesticides as risk factors for Parkinson's disease. Neuroepidemiology 2011; 37(3-4):222-30.
10. *Hertzman C, Wiens M, Bowering D, Snow B, Calne D.*: Parkinson's disease: A case-control study of occupational and environmental risk factors. Am J Ind Med 1990; 17(3):349-55.
11. *Hertzman C, Wiens M, Snow B, Kelly S, Calne D.*: A case-control study of Parkinson's disease in a horticultural region of British Columbia. Movement Disorders 1994; 9(1):69-75.
12. *Firestone JA, Smith-Weller T, Franklin G, Swanson P, Longstreth W, Checkoway H.*: Pesticides and risk of Parkinson disease: a population-based case-control study. Arch Neurol 2005; 62(1):91-5.
13. Office HISD. Occupational disease. Available from: http://www.hiso.or.th/hiso/tonkit/tonkits_17.php.
14. Center NPI. Biomarkers of Exposure: Organophosphates. Available from: http://npic.orst.edu/mcapro/opbiomarkers.html.
15. Diseases BoOaE. Questionnaire for assess the risk of the work of farmers from pesticide exposure. Available from: http://www.envocc.org/downloads/year54/farmer%20assess%20form1_6jan54.pdf
16. *Norkaew S, Siriwong W, Siripattanakul S and Robson GM.*: Knowledge, Attitude, and Practice (KAP) of Using Personal Protective Equipment (PPE) for Chilli-Growing Farmers in Huarua Sub-District, Mueang District, Ubonrachathani Province, Thailand. J Health Res 2010; 24(2):93-100.

17. *Taneepanichskul N, Siriwong W, Siripattanakul S, Pongpanich S and Robson GM*.: Risk assessment of chlorpyrifos (organophosphate pesticide) associated with dermal exposure in chilli-growing farmers at Ubonrachatani Province, Thailand. J Health Res. 2010; 24(2):149-56.
18. *Setthawatcharawanich S, Sathirapanya P, Phabphal K and Limapichat K.:* Short questionnaire for Parkinson's disease as a screening instrument. Clin Neurol Neurosurg 2011; 113(10):885-8.
19. *Mason H.:* The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos. Occup Med 2000; 50(5):343-7.
20. *Wilaiwan W and Siriwong W*.: Assessment of health effects related to organophosphate pesticides exposure using blood cholinesterase activity as a biomarker in agricultural area at Nakhon Nayok province, Thailand. J Health Res 2014; 28(1):23-30.
21. *Simoniello MF, Kleinsorge EC, Scagnetti JA, Mastandrea C, Grigolato RA, Paonessa AM and Carballo MA*: Biomarkers of cellular reaction to pesticide exposure in a rural population. Biomarkers 2010; 15(1):52-60.
22. *Rocca WA, Maraganore DM, McDonnell SK, Schaid DJ.:* Validation of a telephone questionnaire for Parkinson's disease. J Clin Epidemiol 1998; 51(6):517-23.
23. *Hunter CB AL, Nashatizadeh MM, Lay LF, Jankovic J.*: Evaluation of a Parkinson's disease screening questionnaire for use in a community-based setting 2008. Available from: https://www.bcm.edu/departments/neurology/cv.cfm?username=chunter.
24. *Mutch W, Smith W, Scott R*.: A screening and alerting questionnaire for parkinsonism. Neuroepidemiology 1991; 10(3):150-6.
25. *Meneghini F, Rocca WA, Anderson DW, Grigoletto F, Morgante L, Reggio A, Savettieri G and di Perri R*.: Validating screening instruments for neuroepidemiologic surveys: experience in Sicily. J Clin Epidemiol 1992; 45(4):319-31.
26. *Dupont E, Rinne U, Klingler M, Stamm G*.: Parkinson's disease and essential tremor: Differential diagnostic and epidemiological aspects. Parkinson's disease current progress, problems and management, (eds) *UK. Rinne*, *M. Kingler*, G. Stamm, Elsevier/North Holland, Amsterdam. 1980; 165-79.
27. *Chan DKY, Hung W, Wong A, Hu E, Beran R.:* Validating a screening questionnaire for Parkinsonism in Australia. J Neurol Neurosurg Psychiatry 2000; 69(1):117-20.

Received: 03.11.2014
Accepted: 14.01.2015