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Blood Cholinesterase Level and Learning Ability of Primary School Children in an Agricultural Village, Tanjung Karang, Malaysia

Nurul Husna Miswon[1], Zailina Hashim[1*], Vivien How[1] and Raihanah Chokeli[1]

[1]Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.

Authors' contributions

This work was carried out in collaboration between all authors. Author NHM wrote the protocol, managed the literature searches and drafted the manuscript. Author ZH contributed to the research concept, and to the methodological design. Author VH managed the laboratory analyses and experimental process of the study. Author RC managed the field sample collection. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The widespread use of organophosphate (OP) insecticides in paddy fields has led to human exposure. The aim of this study was to determine the relationship between blood cholinesterase concentrations with the children's learning ability in the agricultural village of *Tanjung Karang,* Selangor.
Methods: Seventy-seven primary school children who live less than approximately 1km from paddy fields were selected as the exposed group, while another 62 primary schoolchildren who live more than 1 km away from the agriculture site served as the unexposed group. The questionnaires were completed by the parents. The children's capillary blood was collected using the finger prick technique to determine the blood cholinesterase concentrations using a cholinesterase test kit (Lovibond, AF267, Tintometer Ltd., UK). The McCarthy Scales of Children's Abilities (MSCA) were used to determine the learning ability of these children.

**Corresponding author: Email: zailina@upm.edu.my, husnamiswon@yahoo.com, vivien@upm.edu.my;*

Results: There were significant differences (p<0.05) in blood cholinesterase and all scales in the MSCA between the exposed and the unexposed group. There were significant associations between the blood cholinesterase concentrations and learning ability by all scales in the MSCA (p<0.05). The variable that significantly influenced the blood cholinesterase was gender (p=0.008). Blood cholinesterase was the most significant influencing factor on the learning ability, especially on the motor scale (p=0.002).
Conclusion: Blood cholinesterase concentrations were significantly higher and had a significant relationship with the learning ability of the exposed group, especially the motor performance.

Keywords: Blood cholinesterase; learning ability; schoolchildren.

1. INTRODUCTION

Agriculture is one of the major and important sectors, which best contributes to the overall economic growth and modernization in Malaysia. Agriculture growth and productivity increases are crucial to sustain the economic development. In agriculture, there is a need to use insecticides in order to protect the plantation from any insects, and to improve plant growth. Globally, the first use of synthetic pesticides was in 1940 since when the consumption has continued to increase [1].

Rice production is vulnerable to a great variety of pests due to the ever-changing balance of the rice and pests cycle. The organophosphate (OP) compounds have become the most widely used insecticides available today. About 18 to 20 types of insect species are found in tropical Asian paddy fields, such as rice gall midge, rice bug, rice stem borer, and rice caseworm. Thus far, the mixtures of OP (chlorpyrifos, diazinon, dimethoate, fenthion, Malathion, quinalphos, and trichlorphon) have been used to combat different types of insect during rice production. Blood cholinesterase can serve as an indicator of OP poisoning and their level of activity in blood is also very well documented [2]. Organophosphate is a powerful inhibitor of acetylcholinesterase, which is mainly found in blood. The inhibition of acetylcholinsterase in the nervous system leads to an accumulation of the neurotransmitter acetylcholine at the synapse and myoneutral junction [3]. Children take in more pesticide relatively to body weight than adults and have developing organ systems that are more vulnerable and less able to detoxify toxic chemicals [4].

Human health, especially of children who are a vulnerable group, show that pesticide poisoning can lead to poor performance on tests involving intellectual functioning, academic skills, flexibility of thought, motor skills and memory disturbance, inability to focus attention reaction time, poormanual dexterity and reduced perceptual speed [4]. The excess acetylcholine accumulates in the synaptic terminals overstimulates the nicotinic and muscarinic receptor [5]. The toxic action could interfere with the human neurologic development process from the early stages of development [6]. Therefore, the aim of this study is to determine the relationship between blood cholinesterase concentrations with the learning ability of farm children in the agricultural village.

2. MATERIALS AND METHODS

2.1 Study Design and Sampling

The study was conducted at the agricultural village, *Kampung Sawah Sempadan* (3° 28' 20.455" N, 101° 17' 51.835" W). Seventy-seven farm children whose school and houses were located in this farming village were recruited as the exposed group. The exposed children were believed to be directly or indirectly exposed to pesticides through different exposure pathways. In addition, an unexposed group of 62 children were selected from among those with a similar socio demographic background but did not live in close proximity to the paddy farms (about 5-10km from the study location). The unexposed group was recruited for comparison purposes in this study.

This research was a cross-sectional comparative study whereby the blood cholinesterase concentrations were determined in the laboratory, while the learning ability of the children was carried out at their school.

The name lists of the children were obtained from the school office. The inclusive criteria were normal and healthy schoolchildren, 7-8.5 years old and who have obtained written permission from their parents or guardians.

2.2 Questionnaire

Questionnaires were used to obtain background information on the studied children. The questionnaires, which were completed by their parents, consisted of personal information, socio-demographic, children's activities, parents' occupation and information, exposure history and health status of the children.

2.3 Blood Cholinesterase Test Kit

The exposure to pesticide was measured using individual rapid test cholinesterase test kits (Lovibond, AF267, Tintometer Ltd., UK). The instrument only required a 0.01 ml sample of blood, which could be easily obtained from a finger prick.

2.4 Mccarthy Scales of Children's Abilities (MSCA)

The McCarthy Scales of Children's Abilities (MSCA) were designed to measure learning ability. The MSCA consist of six scales: Verbal, Perceptual-Performance, Quantitative, General Cognitive, Memory, and Motor. The MSCA contains 18 separate tests, which assesses the child's abilities in a variety of crucial areas [7].

The McCarthy Scales are appropriate for children from 2.5 to 8.5 years of age. The content of the tasks was designated to be suitable for both sexes, as well as for children from various ethnicities, regions and socio-economic groups. The materials and questions are game like and nonthreatening. A wide range of behaviour was sampled, and the children proceeded easily through a number of enjoyable activities [7].

2.5 Statistical Analysis

Univariate analysis was performed to summarize the descriptive demographic information of the study population. An independent t-test and Mann Whitney U test were carried out to compare the significant differences of blood cholinesterase concentrations between the farm children and unexposed children. An association test (Spearman-rho test) was applied to determine the association between blood cholinesterase concentrations with the learning ability among the study group. Simple linear regression was carried out to evaluate the relationship of the selected variables that influenced the blood cholinesterase concentrations among the study population.

3. RESULTS

3.1 Socio-Demographics Information

As shown in Table 1, the socio-demographics of the two study groups were similar. The average age of the children was 8 years and they were mostly female. The majority of their parents' education was high school education for both groups. About 80% of the farm children lived near to the paddy farm (less than 1000 metres), whereas, 90% of the unexposed children lived more than 1000 metres away from paddy farms.

3.2 Blood Cholinesterase Concentration

The AChE levels, which are based on the pH colour (colorimetric) for the normal breakdown process of acetylcholine, were used to evaluate the exposure level from the intensive use of the mixture of OP. The presence of acetic acid determines the normal workout between acetylcholine and acetylcholinesterase; otherwise, colour changes due to the accumulation of acetylcholine in the blood specimen.

Table 2 shows that the median for the exposed group was 50.00 and 12.50 for the inter-quartile range. For the control group, 75.00 was the median and 25.00 was the inter-quartile range. There was a significant difference in blood cholinesterase concentrations between the unexposed and the exposed group (p<0.001).

$$Acetylcholine \rightarrow \boldsymbol{Acetic\ acid} + choline \text{---} \quad \text{[Equation 1]}$$

3.3 Learning Ability by MSCA

Result from Table 3 showed that there were significant differences between the verbal (p=0.012), quantitative (p=0.002), perceptual (p<0.001), and general cognitive index (p<0.001), memory (p<0.001) and motor (p<0.001) scores between the exposed and the unexposed group.

3.4 GeneraL Cognitive INdex

Table 4 shows that there were seven categories for the general cognitive ability scores; namely, very superior (≥130), superior (120-129), bright normal (110-119), average (90-109), dull normal (80-89), borderline (70-79) and mentally retarded (≤69). In the exposed group, the majority were in the average category (37.7%) and for the unexposed group; the majority was in the

superior classification. This showed that the unexposed group had a better general cognitive index than the exposed group.

3.4.1 The relationship between blood cholinesterase concentrations with learning ability among the study group

Table 5 shows that there was no significant relationship between the blood cholinesterase concentrations with learning ability for all the scales, such as verbal, perceptual, quantitative, general cognitive index, memory and motor among the respondents for each of the exposed and the unexposed group. However, when all the respondents were combined, there were significant relationships between the verbal (p=0.008), perceptual (p=0.001), quantitative (p=0.019), general cognitive Index (p=0.002), memory (p=0.001) and motor (p=0.005) scales with the blood cholinesterase concentrations.

3.4.2 Selected variables that influenced the blood cholinesterase concentrations among study group

Table 6 shows the results in which selected variables significantly influenced the blood cholinesterase concentrations after adjusting for all the confounders.

3.4.3 Selected variables that influenced the learning ability using McCarthy Scales of Children's Abilities (MSCA) of the study group

Table 7 shows that among all the six McCarthy Scales of Children's Ability, only motor scale showed a significant relationship between the predictor factors of learning ability.

Equation Model II below is a mathematical model for the prediction of the motor scale among the exposed children in which Multiple Linear Regression was used to determine the association between the motor scale and the other influencing factors.

4. DISCUSSION

4.1 Socio-Demography Background

The study involved a total of 139 primary schoolchildren as the exposed and unexposed group. The confounders, such as age, gender, parents' occupation and educational levels, household income, number of siblings were controlled to avoid bias and inaccurate results. The statistics showed no significant difference between the control and the exposed group except for the distance from the houses to the paddy field.

Table 1. Distribution of socio-demographic information among the study groups

Variables		Exposed (N=77)		Unexposed (N=62)	
		N(%)	Mean (SD)	N(%)	Mean (SD)
Age (years)		-	8.0 (1.0)	-	7.8 (0.8)
Body Mass Index (BMI) (kg/m^2)		-	15.0 (3.2)	-	15.0 (3.1)
Household Income (MYR)		-	1200 (500)	-	1500 (350)
Gender	Male	35 (45.5)	-	30 (48.4)	-
	Female	42 (54.5)	-	32 (51.6)	-
Parents' education level	Elementary school	20 (26)	-	12 (19.4)	-
	High school	56 (72.8)	-	48 (78.2)	-
	Tertiary education	1 (1.3)	-	2 (3.2)	-
Distance from house to paddy field (metres)	<100	26 (33.8)	-	1 (1.6)	-
	100-500	19 (24.7)	-	3 (4.8)	-
	500-1000	15 (19.5)	-	2 (3.2)	-
	>1000	17 (22.1)	-	56 (90.3)	-

Table 2. Comparison of blood cholinesterase concentrations between the two groups

Variable	Median (IQR)		*z*	*p*
	Exposed (N=77)	Unexposed (N=62)		
Blood cholinesterase concentrations (%)	50.00 (12.50)	75.00 (25.00)	-6.749	<0.001*

*Mann-Whitney test; *Significant at p<0.001*

Table 3. Comparison of MSCA Scores between the two groups

Variable	Mean (SD)/ Median (IQR)		*t/z*	*p*
	Exposed (N=77)	Unexposed (N=62)		
Verbal[a]	48.83 (7.603)	52.55 (9.654)	-2.540	0.012**
General Cognitive Index[a]	107.88 (14.721)	117.45 (14.914)	-3.787	<0.001***
Motor[a]	59.04 (8.785)	64.90 (7.671)	-4.137	<0.001***
Perceptual[b]	66.00 (15.00)	72.00 (9.00)	-3.930	<0.001***
Quantitative[b]	48.00 (19.00)	58.00 (20.00)	-3.132	0.002**
Memory[b]	42.00 (12.00)	58.00 (22.00)	-4.404	<0.001***

*[a]Independent t-test; **Significant at p<0.01, [b]Mann-Whitney U test; ***Significant at p<0.001*

Table 4. Distribution of general cognitive index (GCI) scores among the study groups

Variables		Frequency (%)	
[a]General Cognitive Index Scores	Classification	Exposed (N=77)	Unexposed (N=62)
≥ 130	Very superior	7 (9.1)	8 (12.9)
120-129	Superior	7 (9.1)	22 (35.5)
110-119	Bright Normal	23 (29.9)	15 (24.2)
90-109	Average	29 (37.7)	13 (21.0)
80-89	Dull Normal	10 (13.0)	4 (6.5)
70-79	Borderline	1 (1.3)	-
≤ 69	Mentally retarded	-	-

Table 5. Relationship between blood cholinesterase concentrations with the learning ability among the study group

Variables	Blood cholinesterase (%)					
	Exposed (N=77)		Unexposed (N=62)		All (N=139)	
	r	p	r	p	r	p
Verbal	0.186	0.106	0.119	0.358	0.223	0.008**
Perceptual	0.085	0.426	0.139	0.282	0.279	0.001***
Quantitative	0.153	0.184	-0.077	0.551	0.199	0.019**
General Cognitive Index	0.178	0.122	-0.034	0.795	0.258	0.002**
Memory	0.153	0.184	-0.028	0.827	0.267	0.001***
Motor	0.100	0.386	0.053	0.682	0.235	0.005**

*Spearman-rho test, **Significant at p<0.01, ***Significant at p<0.001*

Table 6. Selected variables that influenced the blood cholinesterase concentrations among the study group

Selected variable	Coefficient regression, β	*t*	*p*	F	*p*
(Constant)	78.523	17.72	<0.001***		
Gender	-7.762	-2.823	0.005**		
Fathers' education levels	0.046	0.550	0.583		
Mothers' education levels	0.053	0.638	0.525		
Fathers' occupation	0.057	0.685	0.495	7.969	0.005**
Mothers' occupation	0.046	0.546	0.586		
Household income	0.033	0.391	0.696		
Distance from house to paddy field	0.145	1.761	0.08		
Transportation to school	0.046	0.551	0.583		
Body Mass Index (BMI)	0.121	1.467	0.145		

*Multiple Linear Regression method: Stepwise; **Significant at p<0.01; ***Significant at p<0.001*

Table 7. Selected variables that influenced the motor scale among the study groups

Selected variable	Coefficient regression β	*t*	*p*	*F*	*p*
(Constant)	52.237	17.453	<0.001***		
Blood cholinesterase (%)	0.141	3.242	0.001***		
Fathers' education levels	0.097	1.177	0.241		
Mothers' education levels	0.045	0.547	0.585	10.508	0.001***
Fathers' occupation	0.027	0.323	0.747		
Mothers' occupation	0.077	0.929	0.355		
Household income	0.037	0.446	0.656		
Number of siblings	0.010	0.123	0.902		

*Multiple Linear Regression method: Stepwise; **Significant at p<0.01;***Significant at p<0.001*

Model I

Blood Cholinesterase = 78.523 – 7.762 (*Gender*)
R^2 = 0.048 (5% of existing predictor factors fit the model above)

From the model, only gender of the respondents showed significant and inverse correlation with blood cholinesterase, which indicated that the boys had lower cholinesterase concentrations than the girls, and, thus were more exposed to pesticides.

Model II

Motor scale= 52.237 + 0.141 *(Blood Cholinesterase)*

R^2 = 0.071 (7% of existing predictor factors fit the model above)

From the model, blood cholinesterase had a direct relationship with the motor scale in the McCarthy Scales of Children's Abilities (MSCA) among the schoolchildren.

4.2 Blood cholinesterase concentrations

The Blood Cholinesterase Test Kit showed four different blood levels – normal (75-100%), over exposure level (50-75%), serious over exposure level (25-50%), and, lastly, serious and dangerous over exposure (0-25%). From the categories obtained, the levels were normal and serious over exposure to pesticide. From the results, there were significant differences between the exposed and the unexposed group. The exposed group had a higher percentage, which was seriously over exposed compared to the unexposed group in which the majority was normal.

Pesticides can be absorbed rapidly via all routes, such as respiratory, gastrointestinal, ocular and dermal. Oral ingestion is often accidental by children. The organophosphates and carbamates are powerful inhibitors of the acetylcholinesterase found in nervous tissues and erythrocytes. As a result of this enzyme inhibition, the substrate acetylcholine accumulates. The inhibition of acetylcholinesterase in the nervous system leads to an accumulation of the neurotransmitter acetylcholine at synapses, a competitive acetylcholine antagonist, reverses the biochemical abnormalities at the synapses [3].

4.3 Learning Ability by MSCA between the Exposed and Unexposed Group

The MSCA is designed to satisfy the needs for a single instrument to facilitate the measurement of learning ability and evaluate children at a younger age to determine their general intellectual level. The score and indexes, derived from systematic observation of a variety of cognitive and motor behaviour, are provided by the six scales – Verbal, Perceptual-Performance, Quantitative, General Cognitive, Memory and Motor. These measures provide a better understanding of the capability of the children and those with learning disabilities [7]. The MSCA test used was translated to the Malay language and some of the test example was adapted to suit the local context. For example, animals, such as dogs, which are unacceptable to the Malay culture, were substituted with more acceptable animals, such as cats [8].

Past studies that used MSCA as a study tool to determine the relationship between blood lead (BPb) concentrations and cognitive ability among schoolchildren showed significant adverse effects of lead exposure on the mental function [9]. In this study, the studied groups involved

were from 7 to 8.5 years old. There were significant differences between the exposed and unexposed group in all the scales in the MSCA made up of the verbal, perceptual, quantitative, general cognitive index, memory and motor scales. For the unexposed group, a high percentage was found in the superior level while the exposed group was in the average level. Thus, the unexposed group had a significantly better general cognitive score than the exposed group.

4.3.1 Relationship of blood cholinesterase concentrations with learning ability

There was a significant relationship between the blood cholinesterase and MSCA of the children in the study groups. Early concerns about the possible neurotoxicity of OP insecticides for humans derived from rodent studies showed that prenatal and early postnatal exposure to organophosphate was associated with neurodevelopment deficits, and that these effects had been at exposure levels well below the threshold for systemic toxicity caused by cholinesterase inhibition in the brain [10].

Evidence has accumulated over the past decade showing that non-cholinergic mechanisms may play a role in the neurotoxin effect of organophosphate exposure in rodents, involving disruption of neural cell development, neurotransmitter systems and synaptic formation in different regions of the brain [10].

Such developmental disruptions have been associated with later functional impairments in learning, short-term working memory, and long-term reference memory [11]. The higher prenatal organophosphate exposure was associated with decreases in cognitive functioning on two different intelligence scales of urban minority children at 7 years of age [12].

In some cases of cholinesterase inhibitors poisoning, the short- and the long-term chronic neurological and neuropsychological effects of severe poisoning have been shown. In addition to organophosphorus ester-induced delayed neuropathy, neuropsychological sequelae have been reported even when the cholinesterase level returned to the normal level. Language, attention, and mnesic dysfunction have been described, as well as psychomotor, visuomotor and coding skill disturbance, and even irritability, depression and neuropsychiatric symptoms. The more impaired functions were those involved in the information selection, information processing, associative memory, reasoning and abstraction [13].

4.3.2 Selected variable that influence blood cholinesterase concentrations among study group

There were other variables that could influence the blood cholinesterase concentrations among the study groups. The regression results showed that gender and distance from house to paddy field showed a significant relationship with their blood cholinesterase concentrations.

The gender was a factor influencing the blood cholinesterase concentrations of the study group. As the boys play outdoors more often compared to the girls this might influence their blood cholinesterase level. In addition, this may be due to the fact that boys tend to play near the paddy field and get exposed to pesticide which has been cumulatively deposited in the environment, such as soil, water, foliage and in the air.

The children in close proximity to the paddy field had low blood cholinesterase due to direct exposure from the pesticide used thereon. Insecticides are often sub classified by chemical type as organophsphate (OPs), organochlorine, carbamate, and pyrethroid. Cholinesterase inhibiting pesticides, such as the organophosphorus compounds and carbamate used in agriculture to control insects and weeds, affect the surrounding community, especially susceptible and sensitive groups, such as children [14].

A prospective study of children intoxicated with organophosphates before age 3, found that such children had learning impairments and difficulty in restraining and controlling their active behaviour [15]. Other studies found associations between prenatal pesticide exposure with mental and motor development delays, attention and attention deficit hyperactivity disorder problems, pervasive development disorder problems, smaller head circumference and higher blood pressure [12].

Previous research on children in Ecuador, who lived in the vicinity of intense fresh-cut flower farms, reported lower neurobehavioral scores on gross and fine motor skills, and social skills than the communities distant to the flower plantations [16].

4.3.3 Selected variables that influenced the learning ability among the study group

The learning ability among the study group was measured by MSCA, which consist of verbal, perceptual-performance, quantitative, memory and motor scales. The general cognitive index consists of verbal, perceptual and quantitative scales. The multiple linear regressions were used to determine the selected variables that influenced each of the scales. Overall, only the motor scale showed a significant relationship among the predictor factors of learning ability, which was significantly influenced by the blood cholinesterase.

One of the neurological functions for which adverse effects of neurotoxic pesticides have been repeatedly hypothesized is behaviour. Behaviour is the product of various sensory, motor and associative functions of the nervous system, and the hypothesis is that neurotoxic substances can adversely affect one or more of these functions, disrupt learning and memory processes, or cause detrimental behaviour effects [17]. This finding was supported by a previous study, which stated that exposure to pesticide was associated with less optimal cognitive and motor development [18].

5. CONCLUSION

In conclusion, this study suggests that the early chronic OP exposure of farm children has induced the inhibition of cholinesterase. The subtle effects have a significant effect on the learning ability among the exposed children particularly on their motor performance. Although this study is limited by its small sample size, it provides preliminary evidence that children exposed to a mixture of OP in their early life impairs their cognitive performance in terms of their spatial memory, cognitive learning capacity, and motor performance after chronic exposure.

CONSENT

All authors declare that 'written informed consent was obtained from the parents (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

The ethical approval was obtained from the Research Ethics Committee of Universiti Putra Malaysia.

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COMPETING INTERESTS

Authors have declared that there is no competing interests exist.

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