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Blood Cholinesterase Level and Cognitive Functioning among Primary School Children near Paddy Field in TanjungKarang, Selangor

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ABSTRACT

Background: Paddy is the third most widely planted crop in Malaysia after oil palm and rubber. The use of pesticides lead to increase in yields by protecting the plantation from any pest and unwanted plants. The extensive use of insecticide, may affect the human health especially from occupational and environmental exposures. Children are a vulnerable group to exposures of insecticides because of less-developed metabolism and the on-going maturation of their organ systems. Blood cholinesterase activity is the most effective biomarkers to measure the level of exposure for various pesticides. **Objective:** The purpose of this study is to determine the relationship between blood cholinesterase levels with cognitive function of selected primary schoolchildren. **Results:** Highest percentage of schoolchildren had normal (52.7%) exposure. About 92.9% reported watery eyes as signs and symptoms of pesticide exposure. Most of the school children school children were in the average scores (44.6%) of cognitive function test. All scales in the McCarthy Scale Cognitive Abilities (MSCA) showed significant correlation with blood cholinesterase levels ($p < 0.05$). **Conclusion:** There were significant relationship between blood cholinesterase levels with cognitive function in all the MSCA scales. No school children was reported with mentally retarded cognitive function. Finally, gender, mother's education and blood cholinesterase showed significant relationship with memory and motor scales in the MSCA.

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INTRODUCTION

A pesticide is any substance or mixture of substances for a wide array of chemicals intended to kill unwanted insects, plants, mold and rodents (USEPA, 2010). Exposures to pesticides may range from acute to chronic toxicity effects. In 2008, pesticides were the ninth most common substance reported to poison control centres, and approximately 45% of all reports of pesticide poisoning were on children (Roberts *et al.*, 2012).

In order to make agriculture more productive and sustainable for economic growth, pest management is normally used (Gina *et al.*, 2012). Organophosphate is one of the insecticides mainly used in agriculture on crops, residential setting for pest control and for public health protection against vector-borne diseases. It is used in abundance because of its low cost, readily available, less persistent in the environment, and less susceptible to pest resistance (Wessels *et al.*, 2003). Pesticides affect the nervous system by disrupting the enzyme

that regulates acetylcholine which is neurotransmitter.

In the context of children's exposure to this chemical, the health effects is currently a public health concern. Children are known to be more susceptible to the effects of these exposures, as they have higher rates of metabolism, less mature immune systems, and different patterns of activity (Dalvie *et al.*, 2013). Another study (London *et al.*, 2012), stated that toxicity is higher in children because their rapidly developing brain.

According to Kofman *et al.*, (2006), the performance of children exposed to OP pesticides would be poorer in different cognitive and behavioral measures than the children not exposed to OP pesticides. In addition, high levels of OP pesticide are associated with poor performance on the cognitive measures. Findings indicated that there were significant correlations between the OP metabolites concentrations with the cognitive performance of children (NurulHusna *et al.*, 2015; Lizardi *et al.*, 2008).

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MATERIALS AND METHODS

Study location and sampling:

The study was carried out in 2 schools in Tanjung Karang area, namely Sekolah Kebangsaan Dato Manan and Sekolah Kebangsaan Berjaya. Both schools were classified as exposed group because they were located less than 300 meters from the paddy fields. A total of 112 schoolchildren from Year One to Three were selected for the study. The selection of schoolchildren was based on the inclusive criteria; schoolchildren aged between 7 to 8.5 years old, studied, lived and attended schools near the paddy fields. The schoolchildren should be healthy and permissions obtained from their parents to participate in this study.

Data Analysis:

Statistical Package for Social Science (SPSS) Version 21.0 was used to analyze all the data. The descriptive test was used to calculate mean, median, mode and standard deviation. The normality test was used to test for the normality distribution of each data. The first and second objectives were analyzed using descriptive statistical analysis to determine the blood cholinesterase level and cognitive functioning of the children. Spearman-rho correlation was used when the normality. Multiple linear regression tests were used to analyze the relationship between selected variables that influenced the blood cholinesterase level and the cognitive functioning of study schoolchildren.

Instrumentation and procedure:

This study design was a cross-sectional study. Children's cognitive ability was assessed using

McCarthy Scale of Children Abilities (MSCA) test. Blood samples were collected from all children to determine the blood cholinesterase levels. Parent completed a questionnaire on the socio-demographic information, education, pesticides use, house location and their children's medical background and health conditions.

Blood sampling:

Blood samples of 0.01 ml were obtained from the children through finger prick technique. The cholinesterase levels were measured using rapid cholinesterase test kit Model LOVIBOND 412670 AF267. Indicator solutions (Bromothymol Blue) and substrate solution (Acetylcholine Perchlorate) were the reagents used in the rapid test kit.

Results:

Socio-demographic information:

The study samples were made up of 60 girls and 52 boys and all were Malay ethnic. Table 1 shows the distribution of socio-demographic information of the studied schoolchildren. The highest percentage of participation was from Year 2 (38.4%). The majority of the fathers were farmers (66.1%) and mother were mainly non farmers or housewives (59.8%). About 67.9% schoolchildren were sent to school by their parents by motorcycles because the distances from their houses to school were more than 1 km. The houses and schools were mainly located near paddy field. In addition, the practices of washing hands before eating among the children were high and most of them were not allowed to play near the paddy fields area.

Table 1: Distribution of socio-demographics information among the studied schoolchildren.

Variables	Frequency (n)	Percentage (%)
Age (years)		
Year 1	37	33
Year 2	43	38.4
Year 3	32	28.6
Gender		
Male	52	46.4
Female	60	53.6
Father's Education		
Primary school	67	59.8
Secondary school	36	32.1
Tertiary school	9	8.0
Mother's Education		
Primary school	29	25.9
Secondary school	60	53.6
Tertiary school	23	20.5
Fathers' Occupation		
Farmers	74	66.1
Non-farmers	38	33.9
Mothers' Occupation		
Farmers	45	40.2
Non-farmers/ housewife	67	59.8
Transportation to school		
Walk	5	4.5
Bicycle	7	6.3
Motorcycle	76	67.9
Car	24	21.4
Distance from house to		

school (m)		
<100	10	8.9
>100	21	18.8
500-1000	26	23.2
>1000	55	49.1
House Area		
Near to paddy fields	79	70.5
Far from paddy fields	33	29.5
Wash hand before eat		
Yes	89	79.5
No	23	20.5
Play near paddy fields		
Yes	26	23.2
No	86	76.8

N=112

Reported sign and symptom:

Based on Table 2, there were reported sign and symptom of breathing difficulty, watery eye, running nose, headache, excessive sweating and cough. Between of these classifications, breathing difficulty and watery eye were the highest with a percentage of

92.9% respectively. However, 53.6% schoolchildren are having cough in the past 3 months. These symptoms might happen because of pesticide exposure used in the agricultural activity but further examination by doctor is important to confirm the symptoms.

Table 2: Distributions of reported sign and symptom of the studied schoolchildren.

Classification	Frequency (n)	Percentage (%)
Difficulty Breathing		
Yes	3	2.7
No	104	92.9
Not sure	5	4.5
Watery Eye		
Yes	4	3.6
No	104	92.9
Not sure	4	3.6
Running Nose		
Yes	27	24.1
No	82	73.2
Not sure	3	2.7
Headache		
Yes	36	32.1
No	71	63.4
Not sure	5	4.5
Excessive Sweating		
Yes	8	7.1
No	98	87.5
Not sure	6	5.4
Cough		
Yes	60	53.6
No	52	46.4
Not sure	0	0

N=112

Blood cholinesterase levels:

Blood was obtained through finger prick technique. The purpose of taking blood is to measure the exposure level to pesticides. After the blood samples were being analyzed, it was categorized into four exposure classification as shown in Table 3.

Most of the schoolchildren had (52.7%) normal exposure, while another 46 schoolchildren (41.1%) over exposed to pesticide and the remaining 6.3% of the schoolchildren had serious over exposure. None of the respondent was exposed very seriously and dangerously.

Table 3: Blood cholinesterase levels of studied schoolchildren.

Variables	Frequency (n)	Percentage (%)
Blood Cholinesterase Levels		
Normal (>75-100%)		
Over exposure (>50-75%)	59	52.7
Serious over exposure (>25-50%)	46	41.1
Very serious and dangerous over exposure (0-25%)	7	6.3
	-	-

N=112

Table 4: Correlations between blood cholinesterase levels with the cognitive function.

Variables	Blood Cholinesterase (%)	
	r	r
Verbal		
Perceptual-performance	0.395	0.001***
Quantitative	0.345	0.001***
General Cognitive Index	0.262	0.005**
Memory	0.502	0.001***
Motor	0.210	0.026*
	0.549	0.001***

Spearman-rho test

***Significant at $p < 0.001$ **Significant at $p < 0.01$ *Significant at $p < 0.05$ **Correlation between blood cholinesterase levels with the cognitive function:**

Table 4 shows that there were significant relationships between blood cholinesterase levels with cognitive function in all scales namely verbal ($p=0.001$), perceptual-performance ($p=0.001$), quantitative ($p=0.005$), general cognitive index ($p=0.001$), memory ($p=0.026$) and motor ($p=0.001$).

Selected variables that influenced the blood cholinesterase levels:

Multiple linear regression test was used to determine which selected variables significantly influenced the blood cholinesterase levels. From the result obtained, father's education showed significant regression with the blood cholinesterase levels of these school children ($p=0.041$).

Table 5: Selected factors that influenced blood cholinesterase levels.

Variables	B	S.E.	P.R	p-value	95% C.I.
Constant	-0.290	0.892	0.106	0.745	0.787-4.020
Father's education	-0.869	0.869	4.181	0.041*	1.037-5.482
Mother's education	-0.101	0.366	0.075	0.783	0.442-1.852
Housing area	-0.282	0.453	0.388	0.533	0.310-1.833
Wash hand before eat	-0.755	0.518	2.121	0.145	0.170-1.298
Play near paddy fields	0.219	0.496	0.195	0.659	0.471-3.293
Distance from house to school	0.143	0.210	0.464	0.496	0.765-1.740

N=112

B: Regression Coefficient

S.E: Standard Error

95% C.I: 95% Confidence Interval

*Significant at $p < 0.05$ **The selected variables that influenced the cognitive function:**

Multiple linear regression was carried out to determine the factors that influenced the cognitive functioning of study schoolchildren. Among all the 6 scales, only Verbal and Motor scale shows

significant regression with the predictor factors of cognitive functioning. From Table 6, blood cholinesterase levels shows significant regression with the Verbal scale of study schoolchildren ($p=0.008$).

Table 6: Selected variables that influenced the verbal scale.

Variables	B	S.E.	P.R	p-value	95% C.I.
Gender	-0.116	0.420	0.076	0.783	0.391-2.030
Father's education	0.478	0.358	1.781	0.182	0.799-3.254
Mother's education	-0.339	0.330	1.061	0.303	0.373-1.359
House area	0.138	0.471	0.086	0.769	0.456-2.892
Wash hand before eat	0.766	0.520	2.165	0.141	0.776-5.963
Play near paddy fields	-0.500	0.504	0.984	0.321	0.226-1.629
Blood cholinesterase levels	1.135	0.425	7.132	0.008*	1.352-7.153
Constant	-0.673	0.758	0.789	0.374	

N=112

B: Regression Coefficient

S.E: Standard Error

95% C.I: 95% Confidence Interval

*Significant at $p < 0.05$ **Table 7:** Selected variables that influenced the motor scale.

Variables	B	S.E.	P.R	p-value	95% C.I.
Gender	-1.460	0.557	6.870	0.009*	0.078-0.692
Father's education	0.450	0.427	1.112	0.292	0.679-3.622
Mother's education	-1.103	0.441	6.262	0.012*	0.140-0.787
House area	0.388	0.559	0.482	0.488	0.493-4.412
Wash hand before eat	0.446	0.655	0.465	0.496	0.433-5.643

Play near paddy fields	-0.831	0.618	1.806	0.179	0.130-1.463
Blood cholinesterase levels	-3.076	0.606	25.760	0.001*	6.608-71.115
Constant	-0.120	0.886	0.018	0.893	

N=112

B: Regression Coefficient

S.E: Standard Error

95% C.I: 95% Confidence Interval

*Significant at $p < 0.05$

Form the result shown above, gender, mother's education and blood cholinesterase levels had significant relationship with the Motor scale of study schoolchildren. Gender ($p=0.009$), mother's education ($p=0.012$) and blood cholinesterase level ($p=0.001$).

Discussion:

The study involved 112 primary schoolchildren as respondents. Young children might be highly exposed to pesticide because of their curiosity to explore the environment tend to expose themselves to the potential contaminated soil and air. In children the rate of intake of food water, air per unit of body weight are high which might increase the exposure to pesticides (Eskenazi *et al.*, 1999).

Epidemiologic evidence demonstrated associations between early life exposure to pesticides and pediatric cancers, decreased cognitive function, and behavioral problems (Roberts *et al*, 2012). Response to OPs can occur within minutes with mild symptoms such as headache, dizziness, nausea, vomiting, pupillary constriction, and excessive sweating, tearing, and salivation. More severe cases develop muscle weakness and muscle twitches, changes in heart rate, and bronchospasm and can progress to convulsions and coma (Tracey *et al*, 2004).

Acetylcholinesterase is found in red blood cells as well as in nicotinic and muscarinic receptors. To determine the severity and/or the elimination time of OPs poisoning, one should measure cholinesterase in blood, either by measuring plasma pseudocholinesterase (PCE) or by measuring the cholinesterase in erythrocytes (Willemijin *et al*, 2011). The lower the blood cholinesterase levels, indicates high exposure to organophosphate pesticide exposure.

In general, human health research demonstrates that pesticide poisoning can lead to poor performance on tests including intellectual functioning, academic skills, abstraction, flexibility of thought, and motor skills; memory disturbances and inability to focus attention; deficits in intelligence, reaction time, and manual dexterity; and reduced perceptual speed. Increased anxiety and emotional problems have also been reported (Rauh *et al.*, 2011).

Father's educations were divided into primary, secondary and tertiary school levels. The highest percentage was primary school levels and from the findings, it is the most influencing factor that affects the blood cholinesterase levels because at low education levels, most fathers were farmers who

probably could expose their children to pesticides. Exposures might occur through the take-home pesticide residue on clothing and footwear of farmers who were exposed to pesticides at the farm area. This residue can be accidentally ingested when there is no proper hand washing or bathing after they finished working. Besides, another factor that may influence were the house and school locations, which were near to the work area. The pesticides sprayed to the crops might be blown to the schools and houses nearby. Therefore, the exposure level of those who live in the vicinity were assumed to be high. These children may also be exposed while playing near the paddy fields. Most parents did not allow their child to play near the paddy fields.

Gender and mother's education as confounding factors significantly influenced the motor function of the children. Mother's educations are important in developing the children's cognitive ability when they were growing and developing. The highest educational levels of the parents involved in this study were secondary school education. The boys were more active and often played out-door compared to girls and this influencing blood cholinesterase level and cognitive functioning. The higher exposures of pesticide among boys were because they often played near paddy fields.

Poisoning events and chronic exposure to cholinesterase inhibitors, organophosphates (OPs), and carbamates have traditionally been associated with neurotoxic consequences, such as poor neurobehavioral performance in some cognitive domains such as information processing and memory or delayed neuropathy induction (Tapia *et al*, 2005). Neuropsychiatric effects such as impaired memory, confusion, irritability, lethargy, psychosis, and chronic organophosphate-induced neuropsychiatric disorders also have been reported (Kenneth, 2013). Pesticide exposure is commonly associated with deficits in cognitive and psychomotor function. Sheep dippers and nursery workers exposed to OP showed worse performance on one or several tests of cognitive function (Alavanja *et al*, 2004). OP pesticides can also cause chronic neurotoxicity and behavioral impairment (Paudyal *et al*, 2008). Pesticides are neurotoxic similar to lead toxicant as demonstrated by Zailina *et al*, (2008), in which the adverse effects of lead exposure on mental function in children have been demonstrated in intelligent quotient (IQ) and school performance. Besides lead, mercury have been found to have adverse effects on intelligence and cognitive functioning (Tracey *et al*, 2004).

Part of the brain that associated with the learning process (Savannah *et al*, 2014):

- **Frontal Lobe:** The frontal lobe (located behind the forehead) controls personality, but also problem solving, memory, language, judgment and impulse control. The left side of this lobe is more language based, while the right focuses on processes that do not require language. Damage to this area of the brain may affect critical thinking and problem solving skills.
- **Temporal Lobe:** The temporal lobe has many functions in learning, such as organizing information, memory and speech. It has controls in memory retrieval, visual memory and factual memories.
- **Hippocampus:** The hippocampus is involved in the formation of new memories. It does this by creating concepts, and organizing experiences into them. This helps identify contexts of actions and events, and organization of these into a storage system that makes sense to the brain.

Motor nerves carry messages from the brain and spinal cord to muscles, and make the muscles contract. It is important for major body movement such as walking, maintaining balance, coordination, jumping, and reaching. Students with poor motor development might have difficulty with activities such as writing, sitting up in an alert position, sitting erect to watch classroom activity, and writing on a blackboard (Ann *et al*, 2014). Part of the brain that involved with motor functions is cerebellum. Cerebellum or also known as little brain is located at the back brain underlying occipital and temporal lobes of cerebral cortex. About 50% of neuron located in this part. In addition to movement disorders, cerebella patients also demonstrate subtle cognitive deficits, such as an impaired ability to estimate time intervals. Therefore, damage to the nerve cell will disturbed the learning process of children (James, 2014).

Conclusion:

As a conclusion, there was a significant relationship between blood cholinesterase levels with cognitive functioning in all scales of MSCA used in the study. Majority of the studied schoolchildren had normal blood cholinesterase levels. None were exposed to a very serious and danger levels. The IQ of studied schoolchildren, based on GCI scores ranged from very superior to dull normal. No school children reported with borderline and mentally retarded cognitive functions. The father's education level had significant relationship with the blood cholinesterase levels. Finally, blood cholinesterase levels had significant relationship with the verbal and motor scales of MSCA while the confounders such as gender and mothers' education were significantly related to the motor scale.

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