ORIGINAL ARTICLE

ORGANOPHOSPAHTE PESTICIDE MIXTURE EXPOSURE: THE RELATIONSHIP WITH THE MOTOR COORDINATION OF CHILDREN FROM PADDY FARMING AREA IN TANJUNG KARANG, MALAYSIA

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ABSTRACT

Paddy cultivation is one of the widely planted crop in Malaysia. The growth of agricultural activity leads to the use of Organophosphate pesticide to protect the crop. This study is to determine the relationship between the levels of blood cholinesterase with the performance of motor coordination of children living in paddy farming area in Tanjung Karang, Selangor. This cross sectional study was conducted among 683 children from four schools in an agricultural area. Majority of the children have at a family member worked as farmer and was involved with pesticides. A set of questionnaire on the was given to the children to be filled by their parents. To measure their exposure to pesticide, blood cholinesterase levels were measured. Blood samples were taken through finger prick technique and were then analysed using LOVIBOND 412870 AF287. The children were administered with motor-coordination performance test using WHO Neurobehavioral Core Test Battery and McCarthy Learning Ability Scale. Young group children (6-85 years) showed a mean score of 56.66 in motor-coordination test while older group children (10-11)= years) scored a mean of 45.37. There was a significant relationship between blood cholinesterase level and motor coordination performance among the young-group children (r=0.215, p<0.001) and the older-group children (r=0.106, p=0.049). Based on the Linear Regression test results, total household income of family, and mode of transport used were found to have significant relationship with blood cholinesterase level of children in both groups. In addition, blood cholinesterase level and mothers' occupation were found to have significant relationship with the motor-coordination performance of all children.

***Keywords**: Organophosphate, children, blood cholinesterase, motor-coordination*

INTRODUCTION

After many years, field of agricultural is rapidly grown with the aid of technology and government support. The increasing yield in agriculture to escalate economy profit has made agriculture as one of an important source of national income. Hence, it is crucial to ensure, high yield throughout the years. Thus, pesticide such organophosphate is widely used in the agriculture to protect crop from pests. Based on Malaysia Pesticide Act 1974, pesticide was defined as chemicals used to protect crop from being destroyed [1].

Characteristic of Organophosphate

Organophosphates are ester of phosphoric or phosphorothioc acid which can be found in two forms, either sulphur containing form (-thion) or can be in oxygen containing form (-oxon). When compared, oxon form of Organophosphates would have greater toxicity compared to –thion form of organophosphate. Common organo-phopshate pesticides found in field are dimethyl compounds or diethyl compound group attached to phosphorous [2].

Cholinesterase suppression

However, other than its main role as to kill pests, various past studies had proposed several pesticides including organophosphate to have ability to cause neurotoxicity both in humans and animals. The ability of this pesticide as a cholinesterase inhibitor is well understood. Acetylcholinesterase is an enzyme under the family of cholinesterase (CHEs). This enzyme are specialised carboxylic ester hydrolyse that break down esters of choline. Cholinesterase class includes Acetyl-cholinesterase and Butyl-cholinesterase. Acetylcholinesterase is made up from two different proteins domain, which is a large catalytic domain and C-terminal of peptide [3]. Acetyl-cholinesterase with its main role is to hydrolyse neurotransmitter is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system. In both place, acetyl-cholinesterase supposed to hydrolyse acetylcholine into choline and acetate after activation of acetylcholine receptors at the post-synaptic membrane. Briefly, acetyl-cholinesterase activity is responsible to stop or terminate synaptic transmission, preventing continuous nerve firing at the nerve endings [3]. However, with the presence of organophosphate in human body, it would strike neuromuscular junction as the target place to bind with substrate. When these toxic organophosphates reach at the neuromuscular junction, it will phosphorylate acetylcholine, hence, deficit the function and ability of acetyl-cholinesterase to break down the acetylcholine neurotransmitter. This mechanism will cause the accumulation of acetylcholine in

the central and peripheral nervous system, resulting in acute cholinergic syndrome via continuous neurotransmission.

Impairment of Motor-Coordination

Organophosphates pesticides exposures could result in the deficit of neurobehavioral performance [4] due to the inhibition of blood cholinesterase. Neurobehavioral performance test are commonly conducted to measure human performance of visual perception, response speed, manual dexterity, motor and visual coordination, motor steadiness, perceptual motor speed and short term memory capacity [5]. The test batteries have commonly been used to assess neurotoxicity of pesticide exposure among agricultural workers. Individual with histories of toxic exposure to organophosphates were found to have consistent pattern of deficits on measures of motor speed and motor coordination, sustained attention, information processing and learning ability [6-9]. Motor coordination can be explained as the ability of human to control muscle of the body and is usually defined as the ability to coordinate the action of the eyes and hands together in performing precise manipulative movement [10].

METHOD

This cross sectional study was carried out in an agricultural area in Tanjung Karang, Selangor. This area is one of largest paddy farming land in Selangor. This study involved 683 children which comprised 339 children with younger age of 7-8.5 year and 344 children with the older age of 10-11 year, who lived in an agricultural area. Children were recruited from four schools namely (1) Sekolah Kebangsaan Berjaya, (2) Sekolah Kebangsaan Tanjung Karang, (3) Sekolah Kebangsaan Dato' Manan and (4) Sekolah Kebangsaan Sungai Tiram. Parental permissions were obtained and these children fulfilled the inclusive criteria of mentally and physically healthy. Random sampling method was used to select children from name lists provided by the class teachers. A take home questionnaire was given to be filled by their parents on their socio demographic background and health status.

Cholinesterase Test Kit

Blood cholinesterase concentrations were analysed using rapid test cholinesterase kit Model LOVIBOND 412670 AF267. This colorimetric technique kit uses 0.01ml of blood sample collected by finger prick technique. There were two types of reagent used in this test; the indicator solution consisting of bromothymol blue water soluble dissolved in 250ml of deionized water and substrate solution of 0.25g of acetycholine perchlorate dissolved in 50ml of deionized water. This kit uses the concept of pH changes where when the solutions were mixed with blood, the mixture became acidic. The colour appeared were compared to the comparator kit. The result of blood cholinesterase levels were measured as a percentage of the cholinesterase activity.

WHO-Neurobehavioral Core Test Battery

To measure the motor-coordination performance of older-group children, Trail Making test was administered to the children. Trail Making Test is one of the test included in World Health Organisation Neurobehavioral Core Test Battery (WHO-NCTB), widely used in measuring neurobehavioral performance among population exposed to neurotoxin. To ensure the effectiveness of this battery, WHO Guideline of NCTB was followed and researcher was trained to minimize error variance. The room used to conduct the test was suitable and free from distracting noise, and with acceptable lightning.

McCarthy Scale of Children's Ability

The McCarthy Scale of Children's Ability (MSCA) was used to measure the performance of motor-coordination among the younger-group children. This equipment is appropriate to measure the performance perceptual-performance, quantitative, general cognitive, memory and motor of children within the age of 2.5 to 8.5. This scale was created to make it suitable for male and female children as well as from various ethnicity, region and difference socio- economic groups. For the purpose of this study, only the test items related to motor performance were used.

RESULTS

Socio-demographic Information

This study was conducted in four school; Sekolah Kebangsaan Berjaya, Sekolah Kebangsaan Tanjung Karang, Sekolah Kebangsaan Dato' Manan and Sekolah Kebangsaan Sungai Tiram which were located in paddy field areas. Table 1 shows the results on gender, parents' occupation and level of education, the distance between paddy field area and their house, mode of transport used, household income as well as the BMI status of children (Table 1).

Classification of Blood Cholinesterase Levels

Blood cholinesterase concentrations were classified into four categories. Blood cholinesterase ranged from 100-75% categorised as normal, 74.9-50% categorised as over exposed, 49.9-25% as serious over exposed, and 24-0% was categorised as very serious and dangerously over exposed. Based on the results, the majority of younger and older groups were over exposed. In addition, there were 23 young and 29 older children categorised as serious over exposed (Figure 1).

Table 1: Socio-demographic background of children

Variable	Younger (%), n=339	Older,(%), n=344
Gender		
Female	156 (46)	188(54.7)
Male	183 (56)	156(45.3)
Father's Occupation		
Non-Farmer	126(37.2)	137(39.8)
Farmer	213(62.8)	207(60.2)
Mother's Occupation		
Non-Farmer	299(88.2)	271(78.8)
Farmer	40(11.8)	73(21.2)
Father's Education		
High Education	6(1.8)	5(1.5)
STPM	25(7.4)	33(9.6)
SPM	159(46.9)	165(48.0)
PMR	71(71)	55(16.0)
Primary Education	78(78)	86(25.0)
Mother's Education		
High Education	2(6)	2(6)
STPM	36(10.6)	21(6.1)
SPM	187(55.2)	128(37.2)
PMR	59(17.4)	106(30.8)
Primary Education	55(16.2)	87(25.3)
Household Income (RM)		
>3000	29(8.6)	32(9.3)
201-3000	18(5.3)	41(11.9)
1000-2000	157(46.3)	150(43.6)
<1000	135(39.8)	121(35.2)
Distance of House to Paddy Field (m)		
>1000	41(12.1)	80(23.3)
500-1000	37(10.9)	53(15.4)
100 – 500	69(20.4)	78(22.7)
<100	192(56.6)	133(38.7)
Transport		
Car	59(17.4)	61(17.7)
Motorcycle	256(75.5)	218(63.4)
Bicycle	14(4.1)	37(10.8)
Walk	10(2.9)	28(8.1)
BMI		
Underweight	271(79.9)	247(71.8)
Normal	56(16.5)	81(23.5)
Overweight	11(3.2)	12(3.5)
Obesity	1(0.3)	4(1.2)

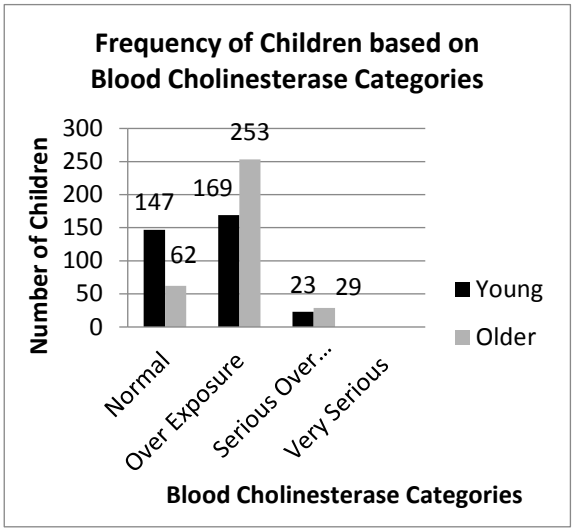

Figure 1: Blood Cholinesterase Categories

Motor-coordination Performance

Table 2 shows the motor-coordination scores among the children. Younger-group children were administered with motor scales of McCarthy Scale of Children's Ability (MSCA) while the older-group children were administered with Trail Making from the WHO Neurobehavioral Core Test Battery (WHO-NCTB)

Table 2: Motor-coordination performance of children

Test	Mean	SD
MSCA Motor Test (n=339)	56.66	9.52
NCTB Trail Making Test (n=344)	45.37	10.65

Relationship between blood cholinesterase and motor coordination performance.

Table 3 shows that there was significant correlation between blood cholinesterase level with MSCA-Motor items ($p<0.001$) and NCTB -Trail Making ($p=0.049$).

Table 3: Relationship between Blood Cholinesterase and motor coordination of children

Test	r	p
McCarthy Motor Test (n=339)	0.215	0.001**
NCTB Trail Making Test (n=344)	0.106	0.049*

*Significant at $p<0.05$, ** Significant at $p<0.001$

Selected variables as predictors of blood cholinesterase level

From the model, it shows that children with the total household family income within the range of RM 1000-2000 had significant relationship with the blood cholinesterase level. In addition, children frequently used motorcycle as mode of transport has 3.168 unit higher risks than children commute by car.

Model I. A significant regression equation found ($F(5.338) = 2.522$, $p<0.001$) with R^2 of 0.022 (2.2% fit the model).

Blood Cholinesterase Level = 61.488 + 4.122 (*income*) - 3.168(*transport)*.

Table 4: Selected Variables as Predictor of Blood Cholinesterase Level

Variable	ß	t	95% CI		p
			Lower	Upper	
Constant	61.488	29.425	57.385	65.591	<0.001
Income(RM)					
> 3000	1				
2001-3000	0.517	0.206	-4.407	5.441	0.837
1000-2000	4.122	2.145	0.348	7.896	0.032*
<1000	1.330	0.681	-2.503	5.163	0.496
Transport					
Car	1				
Motorcycle	-3.168	-2.250	-5.934	-0.403	0.025*
Cycling	-3.986	-1.734	-8.498	0.527	0.083
Walking	-3.364	-1.313	-8.396	1.688	0.190

*Multiple Linear Regressions Test. Method: Enter. *Significant at p<0.05*

Selected Variable as Predictor of Trail Making Test

Model I. A significant regression equation found ($F(5,338) = 3.829$, $p=0.002$) with R^2 of 0.054 (5.4% fit the model).

Trail Making Test Performance = 36.389 + 0.127 (*Blood cholinesterase Levels*) – 4.683 (*mother's occupation*).

From the model, it shows that for every unit increase in Trail Making test, blood cholinesterase level will increase by 0.127 units In addition, children with mother working as farmer would score 4.683 unit lower than children from non-farmer mother (Table 5).

Table 5: Selected Variables as Predictor of Trail Making Test

Variable	ß	t	95% CI		p
			Lower	Upper	
Constant	36.389	11.117	29.951	42.828	0.000
Blood Cholinesterase Level	0.127	2.453	0.025	0.228	0.015*
Income(RM)					
> 3000	1				
2001-3000	4.406	1.773	-0.483	9.295	0.077
1000-2000	3.378	1.649	-0.651	7.408	0.100
<1000	1.886	0.903	-2.221	5.994	0.367
Mother's Occupation					
Non-Farmer	1				
Farmer	-4.683	-3.303	-7.471	-1.894	0.001*

*Multiple Linear Regressions Test. Method: Enter. *Significant at p<0.05*

Selected Variable as Predictor of McCarthy Motor Coordination Test

From the model, (Table 6) it shows that for every unit increase in MSCA Motor Test score, blood cholinesterase level increased by 0.135 units.

Table 6: Selected Variables as Predictor to the MSCA Motor Test

Variable	ß	t	95% CI		p
			Lower	Upper	
Constant	47.924	21.587	43.557	52.291	0.001*
Blood cholinesterase level	0.135	4.040	0.069	0.201	0.001*

*Simple Linear Regressions Test. Method: Enter. *Significant at p<0.05*

Model I.
A significant regression equation found (F (1,337) = 16.324, p<0.001) with R^2 of 0.215 (21.5% fit the model).

MSCA = 47.924 + 0.135 (*blood cholinesterase levels*)

DISCUSSION

Socio-demographic background

This cross sectional study was conducted in four schools located from the paddy field areas. There were 156 (46%) of female children in young-group children involved compared to 183 (56%) of male children. On the other hand, number of female children was higher than the male in older-group children, where 188 (54.7%) were female children and 156 (45.3%) were male children. Gender was measured as a variable as to determine if it would have significant influence on the blood cholinesterase levels and the motor coordination performance.

Based on the survey, most of children in younger and older-group had father working as farmers. However, most of their mothers were not involved in the farm activities and were housewives. Based on the questionnaire, majority of the parents in both group had secondary education.

As for the BMI status, due to young children range of age, both groups of children had lower BMI than the standard. In this study, BMI status was included because the dose of pesticide per body weight is likely to be larger in children and they have a less capacity to remove or detoxify xenobiotic compare to adults [11].

Classification of Blood Cholinesterase

Based on the results, which showed that 169 out of the 339 children in younger-group children had blood cholinesterase level in the range of 74.9-50%, which means they were over exposed and 23 children in the same group have serious over exposure. In addition, 253 out of 344 children in older-group children were found to be in serious over exposure while 29 children were seriously over exposed. This result showed, there was a deviation in blood cholinesterase level from the normal range (100-75%) which indicated that children in this agricultural area would have significant exposure to cholinesterase inhibitor substance. Acetyl-cholinesterase is found in red blood cell as well as in nicotinic and muscarinic receptors. To determine the severity and/or the elimination time of organophosphate, the blood cholinesterase levels in the red blood cell must be measured [12]. The lower the blood cholinesterase levels, indicated high exposure to organophosphate pesticide [8] .

These findings were consistent with the study by Miswon et al, (2015), where children live in agricultural area had lower blood cholinesterase level, compared to children who live in non-agricultural area [9]. Study by Lu, (2007), showed also that children with organophosphates exposure had significant decrement in cholinesterase activity due to the presence of cholinesterase inhibitor in red blood cell [13].

Motor-coordination Performance

Assessment of motor-coordination function was conducted to observe the performance in motor-function among the children. The NCTB and MSCA had been administered on various children according to their age. The results showed that children in both groups showed poor performance in motor coordination. Children had poor performance in motor coordination and low rapid coordination of eye, and hand indicated they have low focus of attention problem. This study was consistent with the result proposed in a previous study where children exposed to organophosphate pesticide had a deficit in inhibitory motor control [15].

Relationship between Blood Cholinesterase with Motor Coordination Performance

Based on the result, it showed that there was significant relationship between blood cholinesterase and motor coordination function in both groups of children. A study reported an epidemiological evidence which demonstrated association between early life exposure to pesticides and paediatric cancer, decreased cognitive function, and induced behavioural problems[15]. Non-cholinergic mechanism 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 [16]. It was reported that, the main neurotoxin reaction after absorption of cholinesterase inhibitor is acute cholinergic syndrome due to the inhibition of acetyl-cholinesterase enzyme which could lead over the stimulation of acetylcholine in synapse [17]. In long term of organophosphate exposure, the loss of enzyme function allows accumulation of acetylcholine peripherally at the cholinergic neuro-effector junction (muscarinic effects), skeletal nerve muscles and autonomic ganglia (nicotinic effect) as well as the central nervous system, in which, high acetylcholine concentration can cause sensory and behavioural disturbance, incoordination, depressed motor function and respiratory depression [18]. This study was consistent with several previous studies which found significant correlations between the present biomarker with the Digit Span and Trail Making Test, and McCarthy Learning Ability Scale [5,9,19].

Selected Variables as Predictor of Blood Cholinesterase Level

In this study, income status and mode of transport used were found to have significant influences on blood cholinesterase levels. Based on the multiple linear regression test, children from the family

with total household income within RM1000-RM2000 had significant influence on the blood cholinesterase level. This explains that children with low family income would possess greater risk of having lowered blood cholinesterase than those with higher family income.

Low income could be the reason for parents involved with agriculture activities, failed to provide adequate measure for pesticide storage as proper storage of pesticides is less important. This could increase the exposure of pesticides to children with family member working in agriculture. Another variable which showed significant influence was the mode of transport. Result showed, children commute with motorcycle had significant risk of having low blood cholinesterase level compared those who used car. In addition, motorcycle vehicle was found to be the most frequently used in the agricultural area. Based on the past studies, vehicle used for work used to transport family, also contributed to the take home pathway exposure. Numerous studies had demonstrated that children of exposed worker have significantly higher exposure to workplace chemical than the control children [20-22].

Selected Variables as Predictor of Motor-Coordination Performance

Based on the result, it showed that the blood cholinesterase level had significant influence on the motor coordination performance in both groups of children. Acetylcholinesterase terminates signal transmission in cholinergic neurons by catalysing the hydrolysis of the neurotransmitter acetylcholine, which allow this neurons to return to the resting state after activation. Inhibition of acetylcholinesterase, due to organophosphates exposure, lead to the accumulation of acetylcholine at the synapse, resulting in overstimulation of cholinergic neurons which impaired the motor functions [23].

Mothers' occupation also had significant influence on the motor-coordination performance of older-group children. Children with mothers who were farmers would have more risk to pesticide exposure than the children with non-farmer mothers. If parents or other family members work with pesticides, chemical were brought into the home by the work boots, tools, work clothing, or even through the vehicles they used to go to work. These findings were consistent with previous studies which found that children with parents who were farmers, have significantly greater exposure to workplace chemical than the comparative children [20-22].

CONCLUSION

As a conclusion, the total household income and the mode of transport used were found to have significant influence on the blood cholinesterase level on both groups of children. As a result, the blood cholinesterase level and mothers' occupation were found to have significant influence on the impaired motor-coordination of these children.

ABBREVIATION

WHO-World Health Organisation, NCTB-Neurobehavioral Core Test Battery, CI-Confidence Interval.

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