

The Effect of Pesticide on the Activity of Serum Cholinesterase and Current Perception Threshold on the Paddy Farmers in the Muda Agricultural Development Area, MADA, Kedah, Malaysia

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Summary

Background: The aim of this study was to find out the indicator as a marker of person who are exposed to pesticides (organophosphate). Up to now the serum cholinesterase is used as a marker. This new method will not taking blood vein since this be the obstacle in conducting research in a rural area.

Methods: A cross sectional study was conducted to investigate the effect of pesticide exposure on the activity of serum cholinesterase (ChE) and current perception threshold (CPT). The samples are 60 paddy farmers (exposed group) and control group taken from the office of Muda agricultural area of Kedah is 19 do not expose to pesticide. The CPT values measured using a portable constant current electric nerve stimulator, Neurometer CPT/Eagle (Neurotron Incorporated, Baltimore, USA). Assessments carried out by one examiner on the index finger (median digital nerve) and the great toe (peroneal digital nerve). The current used are 3 neuroselective frequency range 2000Hz, 250Hz, and 5Hz. The serum cholinesterase activity measured spectrophotometrically using cholinesterase inhibition test kit CHE MHE 1,144729¹

Result: It was significantly evident that the serum cholinesterase activity noted reduced among the paddy farmers ($p=0.014$). The CPT values were significantly elevated for the 2000Hz frequency range for both the measurement sites (index finger $p<0.0001$ and great toe $p<0.0001$). For the 250Hz frequency range CPT values were significant only for the index finger ($p=0.012$). However there was no significant difference for the 5Hz frequency range. There was also a significant correlation (negative) between CPT values and serum cholinesterase activity more for the 2000Hz frequency range (index finger $r=0.672$, $p<0.0001$ & for great toe $r=0.736$, $p<0.0001$). The results were suggestive of subclinical impairment of distal axonopathy considering the fact only 25% of the farmer showed clinical manifestation of numbness.

Conclusion: The selective involvement of the large diameter sensory fibres (250Hz and 2000Hz) were reflective of toxic peripheral neuropathy. Neurometer CPT/Eagle used as indicator for detecting the effect of pesticide instead of using cholinesterase enzyme activity.

Key Words: Serum cholinesterase, Current perception threshold (CPT), Distal axonopathy, Neuropathy, Pesticides, Paddy farmers

This study was carried out in the area where most of the people are paddy farmers in Kedah, Northern part of Malaysia. This area is the main source of rice production for Malaysia (rice bowl of Malaysia). The number of the farmers under MADA administration are 63,000 farmers.

MADA, Muda Agriculture Development Authority is semi government body and well organises Agriculture Organisation. This area produces rice for Malaysia consumption and has a very good irrigation system. Most of the activity in paddy farming already uses mechanical system, such as irrigation and harvest. The big problems are the methods in using pesticides by the farmers. Due to the tropical climate and level of education of the farmers, the protection gear is not suitable to use in the field. Most of the farmers have exposed to the pesticide between 5 to 20 years. The samples for this study are sprayer.

Malaysia with tropical climate (Peninsular Malaysia has a tropical climate with high humidity $\pm 95\%$. Temperature range from 24° - 32°C with an average rainfall of about 100 inches a year. It is not easy to use personal protection (gloves, mask) while expose to pesticide in the tropical climate.

The purpose of this study is to find out the markers to monitor the person who was expose to pesticides. Usually as an indicator we used the enzyme cholinesterase level to detect the person in danger or not. We took 3,500U/I as the cut off point as a dangerous level. Anyway withdrawal of 10 cc of blood from the farmers is not that easy. By using Neurometer, we do not need to withdraw blood for detecting the level of cholinesterase. We can detect the person who was expose to pesticides by measure the nerve conductive velocity. Neurometer is easy to manage and can be run by technologist. It will save much budget working in the field and the study can cover a big number of sample.

Materials and Methods

Study Population

Paddy farmers in Muda area, Kedah, Malaysia.

A cross sectional study was conducted in Kedah, Northern part of Malaysia and border with Thailand MADA area consist of:

1. Kota Sarang Semut
2. Kuala Perlis
3. Pendang and
4. Jitra

The cluster random sampling employed and the number of the sample is 60 farmers, and 19 non exposed to pesticide as control, so the totals of the sample are 79 persons. The case subjects (the 'exposure' group or sprayer) exposed to pesticide between 5 to 20 years. The control group were the person who was not farmer and all of them are from the office of MADA in Kedah (Typist, Administration and office boy). We do not match the age between samples and control. The matching is impossible, since the age of the retirement in the MADA office is 55 years. That is why the mean samples lower than the mean of the control group.

The nerve conduction was measure using CPT (Current Perception Thresholds used as a measure of the integrity of the peripheral nervous system. This new diagnostic technique is quick, simple to perform, non invasive and non aversive and provides a sensitive quantitative measure of sensory function. This diagnostic stimulator will be useful for screening farmers who exposed to organophosphate and entrapment neuropathies in which sensory impairment is an early finding. The CPT is a portable battery-operated transcutaneous electrical nerve stimulator (Neurometer, Neurotron Inc., 6211 Falls Rd, Baltimore, MD 21209). It emits a graded sinusoid at 5,250, and 2000Hz at digitally calibrated levels from 0 to 10 mA. The current was delivered to the skin surface by a pair of 1-cm diameter standard carbon electrodes separated by 1.7-cm. A thin covering of standard electrode paste used as a conducting medium. At each frequency, the current incremented over variable time intervals until the subject could perceive a sensation at the site of stimulation. The current was then decremented and reincremented until the same threshold measure obtained on at least three consecutive trials. In this study the current perception threshold examination consisted of stimulation at the dorsal surface of the distal phalanx of the index finger and great toe.

ChE or cholinesterase in this study is serum ChE and this study we use Kit cholinesterase CHE MHE 1 1447297 (Boehringer Mannheim).

Results

The sample size is 88 and, where 66 farmers and 22 controls. Nine samples excluded, due to the diseases (6 farmers and 3 controls).

Farmers age between 28 to 72 years (mean 44.6 ± 9.6) and controls between 22 to 50 years (mean 34.2 ± 7.6)

Mean cholinesterase activity farmers and controls are 4479.20 ± 1127.64 and 5160.75 ± 598.99 respectively (Fig. 1). This data shows that cholinesterase activity among farmers is lower than control. Since Malaysia do not have normal value for cholinesterase activity, we took 3500U/I as a cut off point¹. Using this cut off point, 25% farmers will be classify as low cholinesterase activity. The percentage of controls is 84 % with concentration of cholinesterase between 4500 and 6250U/I. This follows the result study in 1994 by Jamal, Nor Hashim and Syarif also in MADA. Two studies find out 29% farmers with cholinesterase less than 3500U/I^{2,4}.

CPT value for median nerve among farmers compare to the control was significant different, with 5Hz < 0.05, 2000Hz (p=0.0001) and 250Hz (p= 0.012).

Table I shows the result CPT value for median nerve by frequency between farmers and control.

Table II shows CPT value for peroneal nerve by frequency between group. For this nerve only 2000Hz significant with p=0.0001, and 250 and 5Hz not significant with p>0.05. Fig.1 shows the mean cholinesterase activity farmers lower than control.

The correlation between serum cholinesterase activity and CPT value, shows by Table III and Table IV.

From the value of r (correlation coefficient) the data can tell, if cholinesterase activity lower, the CPT value will increase. The correlation between CPT value and ChE activity for the peroneal nerve at frequency 2000Hz is stronger compare to the other frequency (250 and 5Hz). The result also follows by median nerve at the same frequency.

Conclusion

1. Serum cholinesterase activity is lower among farmers exposed to pesticides compare to control group p<0.05.
2. CPT (Current Perception Threshold) for median and peroneal nerves at 2000Hz both significant p=0.0001. At 250Hz only median nerve shows significant p<0.05 and using frequency 5Hz both nerves show non significant.
3. CPT value increase at 2000Hz and 250Hz shows that it is neuroselective for peripheral sensory nerve type A, and possibility to indicate the polineuropathy toxicity.

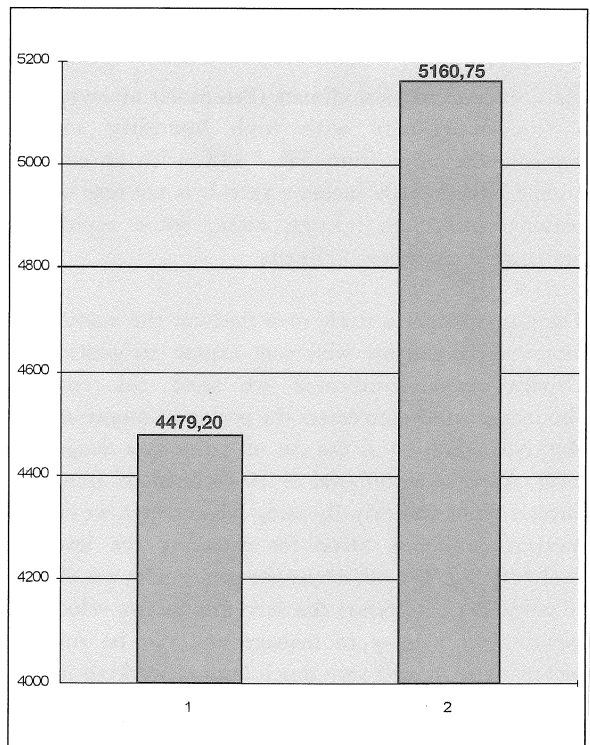


Fig. 1: Comparison mean Cholinesterase activity between famers and Controls (U/I)

Table I
CPT Value for Median Nerve at Frequency between Group (Farmers and Control)

Frequency (Hz)	Farmers N=60		Control N=19	
	Mean	s.d.	Mean	s.d.
2000	389.05	100.39	298.63	79.34
250	164.43	50.24	138.37	32.99
5	87.63	33.07	78.05	15.97
2000Hz: p < 0.000		250Hz: p < 0.05		5Hz: p < 0.05

Table II
CPT Value for Peroneal Nerve by Frequency Between Group (Farmers and Control group)

Frequency (Hz)	Farmers N=60		Control N=19	
	Mean	s.d.	Mean	s.d.
2000	473.03	118.91	369.05	73.63
250	189.80	61.06	184.29	38.92
5	93.43	33.54	101.16	26.49
2000Hz: p < 0.0001		250Hz: p > 0.05		5Hz: p > 0.05

Table III
Correlation Coefficient Between CPT Value and ChE Activity by Frequency (Hz) for Median Nerve Among Farmers

Frequency (Hz)	Coefficient Correlation r	P
2000	- 0.672	0.0001
250	- 0.436	0.0001
5	- 0.532	0.0001

Table IV
Correlation Coefficient Between CPT Value and ChE Activity by Frequency (Hz) for Peroneal Nerve

Frequency (Hz)	Coefficient correlation r	P
2000	- 0.736	0.0001
250	- 0.473	0.0001
5	- 0.383	0.003

4. There is negative correlation between Current Perception Threshold (CPT) value with Cholinesterase activity level among farmers exposed to pesticides $r=-0.672$, ($p=0.0001$) for median nerve and $r=-0.763$ ($p=0.0001$) for peroneal nerve.
5. Increase in CPT value may not be only due to cholinesterase inhibiting pesticides. Therefore, we have to consider other pesticides such as organochlorine and 2, 4 D. For this purpose further studies regarding these pesticides have to do in the future.
6. For detection of pesticides exposed among farmers we can use CPT as markers. Serum cholinesterase level still can use as a marker with blood withdrawal. Anyway, blood withdrawal is one of the limitation of the study.

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References

1. Heil W, Schucklieb F, Zawta B. Reference range for adults and children: Pre-analytical Consideration. Boehringer Mannheim Diagnostic System, Germany 1993.
2. Futagami K, Otsubo K, Nakoa Y, Aoyama T, Iimori E, Urakami S, Ide M, Oishi R. Acute organophosphate poisoning after disulfoton ingestion. *J. Toxicology. Clin Toxicology* 1995; 33 (2): 151-5.
3. Jamal HH, Noor Hashim I, Syarif HL, Syed Mohd SA. and Norazah D. Health effect related to pesticides use among paddy farmers of Muda area in Kedah. Malaysia. *Rice Agroecosystem of the Muda Irrigation Scheme, Malaysia* 1998; 14: 181-91.
4. Markowitz SB. Poisoning of an urban family due to misapplication of household organophosphate and carbamate pesticides. *Clinical Toxicology* 1992; 30(2): 295-303.
5. Rama DB; Jaga K. Pesticide exposure and cholinesterase level among farm workers in the Republic of South Africa. *The Science of Total Environment* 1991; 122 : 315-9.
6. Steenland K, Jenkins B, Ames RG, O'Malley M.; Chrislip D, Rosso J. Chronic Neurological sequelae to pesticide poisoning. *American Journal of Public Health* 1994; 84 (5): 731-6.
7. American Academy of Neurology. Therapeutics and Technology Assessment Subcommittee submission for the evaluation of the Current Perception Threshold (CPT) Quantitative Sensory Test (QST). 1996.
8. Katims JJ; Rouvelas P; Sadler BT; Weseley SA. Current Perception Threshold. Reproducibility and Comparison with Nerve Conduction in Evaluation of Carpal Tunnel Syndrome. *ASAIO TRANSACTION*, July-September 1989; 35 (3).