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Research article

ACETYLCHOLINESTERASE LEVELS IN FARMERS EXPOSED TO PESTICIDES IN MALAYSIA

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ABSTRACT: Agriculture is an important component of the Malaysian economy. Pesticides are widely used by farmers to increase crop production. Acetylcholinesterase (AChE) is known to play an important role in the degradation of acetylcholine (ACh) at the neuromuscular junction of the nervous system. The purpose of this study was to determine the effect of pesticide exposure on serum levels of AChE of farmers. A cross-sectional study was conducted. A total of 95 farmers from Kelantan (n = 49) and Selangor (n = 46) aged between 23 and 71 years were recruited. AChE concentration was measured by spectrophotometry. The results of this study showed that the mean AChE concentrations in farmers from Kelantan and Selangor were 2,715 and 2,660 U/L, respectively, significantly different ($p < 0.05$) from normal reference value (3500 U/l). Pearson correlation test showed a moderate correlation between AChE level and age ($r = -0.551$) and a strong correlation between AChE level and working period ($r = -0.872$) in farmers in Kelantan. AChE levels in Selangor were also moderately correlated with age ($r = -0.353$) and working period ($r = -0.515$). In conclusion, increasing age and long-term pesticide exposure reduce AChE levels in farmers.
Key words: Pesticides, acetylcholinesterase, farmers, Malaysia

INTRODUCTION

Pesticide consumption has markedly increased with Malaysia's need to increase food production to feed its increasing population and the rapid growth of its cash crop industry. While Malaysia is moving towards industrialization, agriculture remains a significant component of its economy. In fact, most multinational agrochemical companies are actively targeting the expanding markets in these developing countries. Therefore, it is important for health scientists in developing countries like Malaysia to monitor the health impacts of increasing pesticide use on their farmers. A variety of modern agricultural technologies have been introduced, namely ploughs, harvesters, pesticides, fertilizers, and lime (Fuad et al. 2012). Previous studies indicated that agricultural pesticide application can reduce pest-induced plant damages by approximately 30% (Jamal et al. 2000). Among 893 cases of pesticide poisoning reported by government hospitals throughout Malaysia, 278 were fatal. In 1993, the number of pesticide poisoning cases increased to 1,270, with 394 deaths. Pesticide exposure was associated with a variety of common symptoms of chemical poisoning, ranging from mild effects such as skin irritation, nausea, and vomiting, to even death. A study conducted in Malaysia reported that 43% of pesticide users experienced symptoms such as headache, drowsiness, vomiting, breathing difficulties, and skin and eye irritation (Jamal et al. 2000). Cholinesterase, more commonly referred to as acetylcholinesterase, is an enzyme essential for normal functioning of the nervous system of humans, birds and insects. Acetylcholinesterase inactivates acetylcholine, the chemical messenger normally active at the junctions between nerves and muscles, between many nerves and glands, and at the synapses between certain nerves in the central nervous system. When cholinesterase levels are low because of excessive inhibition, the nervous system can malfunction, which can lead to death (Frederick 2009). Certain chemical families of pesticides, such as organophosphates and carbamates, work primarily against arthropod pests by interfering with or inhibiting cholinesterase (Eckert et al. 2006). While the effects of cholinesterase-inhibiting products are intended for pests, these chemicals can also be poisonous to humans. Agriculture workers in open fields, pesticide industry workers, and exterminators have increased risks of pesticide exposure (Christos and Illias. 2011).

Growing concern over the impact of the escalating use of pesticides in the Malaysian agricultural industry on public health and safety prompted this study, which was carried out in Pasir Puteh and Bachok, Kelantan as well as Tanjung Karang, Selangor. This study collected baseline information about health effects, namely symptoms of pesticide exposure and acetylcholinesterase (AChE) inhibition, in Malaysian paddy farmers exposed to herbicides and insecticides used in paddy farming.

METHODOLOGY

This cross-sectional study included 95 farmers between 18 and 72 years of age from Pasir Puteh and Bachok, Kelantan and Tanjung Karang, Selangor, Peninsular Malaysia. Purposive sampling was used to select locations, whereas simple random sampling was used to select subjects. Farmers were interviewed using guided questionnaires to obtain information about pesticide use and the exposure symptoms they had experienced during and after pesticide applications. After the interview, each participant underwent a general medical examination. During this examination, 10 ml of venous blood was collected from each farmer for acetylcholinesterase testing. The blood samples were collected in plain tubes and centrifuged at 5,000 rpm for five minutes to separate the serum.

The serum samples were transported back to the laboratory at 0-4°C, where they were stored below 0°C until analysis.For the determination of AChE level, a substrate of acetylthiocholine and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in the form of powder left at room temperature after removed from the refrigerator. DTNB and Acetylthiocholine powder weighed and each of it was dissolved in Phosphate Buffer Saline (PBS) and distilled water (Dhingra et al.2011).3 ml DTNB and 20 µl serum were added in test tube. To this, 100 µl of Acetylthiocholine was then added. The AChE in the serum will then hydrolyze acetylthiocholine to thiocholine and acetyl acid. Thiocholine will then react with DTNB to form a colored compound, 2-nitro-5-mercaptobenzoate.

The light absorbance of 2-nitro-5-mercaptobenzoate was then measured with an ultraviolet-visible spectrophotometer at a wavelength of 405 nm against air (blank), with a light path of 1 cm. Absorbance was read at intervals of 30, 60, and 90 seconds. The average absorbance change over 30 seconds was then multiplied by a constant, 22710, to obtain the serum AChE level in international unit per liter (U/L) at 25°C. Data were statistically analyzed by using SPSS version 20.0.

RESULTS

A total of 49 and 46 farmers from Kelantan and Selangor, respectively, were included in this study (Table 1). Among these, 45 farmers in Kelantan were male, whereas 4 were female; in Selangor, 43 farmers were male and 3 were female. Most of the farmers in both places reported working periods ranging between 6 and 20 years.The mean AChE levels in farmers in Kelantan and Selangor were 2,715 and 2,660 U/L, respectively. The AChE levels were significantly lower than the normal value (3,500 U/L, $p < 0.05$).

Table 2 shows AChE levels according to the types of pesticides used by farmers in Kelantan, whereas Table 3 shows AChE levels according to the types of pesticides used by farmers in Selangor. Most farmers in this study used organochlorine and carbamate. The AChE levels in farmers in Kelantan differed significantly among those who used pesticidescontaining organochlorine and carbamate ($p < 0.05$), while the levels in farmers in Selangor differed significantly among those who used carbamate ($p < 0.05$). As shown in Table 4, there was a negative correlation between AChE level and age and working period.

Table No-1: Demographic Data

Parameter	GROUP	Kelantan46 (%)	Selangor49 (%)
Sex	Male	45 (92%)	43 (93%)
	Female	4 (2%)	3 (7%)
Age	18-40 years	2 (4%)	8(17%)
	41-64 years	36 (74%)	37(80%)
	>65 years	11 (22%)	1 (3%)
Working Period	1-5 years	2(4%)	6 (13%)
	6-20 years	15 (57%)	11(54%)
	21-40 years	15 (31%)	11 (24%)
	41-60 ears	6(8%)	4 (9%)

Total number of subjects from Kelantan (46%) and Selangor (49%)

Table No-2: Difference of AChE level according to the type of pesticide used by farmers in Kelantan

Type of Pesticide	Level of AChE(U/l) (Mean ± Standard Deviation)		t	p
	Yes	No		
Organochlorine	2423 ± 576 (n=25)	3030 ± 580 (n=24)	-3.549	0.001*
Organophosphate	2754 ± 529 (n=11)	2704 ± 688 (n=38)	0.244	0.809
Carbamate	2433 ± 586 (n=26)	3033 ± 589 (n=23)	-3.569	0.001*
Pyrethrin	2426 ± 845 (n=10)	2357 ± 1199 (n=39)	-0.175	0.862
Pyrethroid	2151 ± 1005 (n=1)	2391 ± 1130 (n=48)	-0.357	0.723

*AChE level is significant (p<0.001) with the use of pesticide

Table No-3: Differences in AChE level according to the type of pesticides used by farmers in Selangor

Type of Pesticide	AChE level (U/l) (Mean ± Standard Deviation)		t	p
	Yes	No		
Organochlorine	2766 ± 563 (n=20	2579 ± 615 (n=26)	-1.057	0.296
Organophosphate	2643 ± 624 (n=9)	2664 ± 595 (n=37)	0.095	0.925
Carbamate	2894 ± 629 (n=22)	2446 ± 479 (n=24)	-2.729	0.009*
Pyrethrin	2870 ± 619 (n=5)	2635 ± 594 (n=41)	-0.834	0.409
Pyrethroid	2722 ± 1510 (n=2)	2657 ± 562 (n=44)	-0.148	0.883

* Ache level is significant (p <0.001) with the use of pesticides

Table No-4: Correlations between levels of AChE with age and working period

	AChE level	
	Kelantan	Selangor
Age	r= -0.551 p<0.001	r= -0.353 p=0.023
Working period	r= -0.872 p<0.001	r= -0.515 p<0.001

Negative correlation between AChE level and age and working period

DISCUSSION

Acetylcholinesterase (AChE) is important for normal functioning of the nervous system in vertebrate animals including humans and birds, as well as in insects. In human system, AChE hydrolysis acetylcholine is normally active at the junction between nerves and muscles, between nerves and glands, and in the synapses between nerves in the central nervous system. Acetylcholinesterase is used to monitor and diagnose organophosphate and carbamate exposure in humans (Osten et al. 2004; Barry et al. 2005).

In this study, serum AChE levels in farmers were below the normal range of 3,500 U/L. The findings of this study support those of previous studies by Jamal et al. (1995) and Lubis et al. (2002), which concluded that low levels of AChE in farmers were due to pesticide usage. Ueyamaa et al. (2009) reported that organophosphate is widely used to increase crop productivity, similar to reports from the farmers in this study regarding their use of organophosphates. Organophosphates prevent AChE from functioning at neurons (Vidair 2004), and the effects of organophosphate toxicity are due to AChE inhibition (Eckert et al. 2006). However, use of organophosphates did not appear to affect AChE levels in farmers in Kelantan and Selangor in this study. This might be due to the restoration of AChE levels after inhibition, shown to occur following organophosphate detoxification and cleansing from the body (Kazi and Oommen 2012). AChE levels return to normal owing to increased synthesis of new enzyme catalysts, but this response takes a significant amount of time (Kaufer et al. 1999).

Carbamate is an insecticide widely used in agriculture to protect crops from pests (Simonians et al. 2005). AChE levels were significantly lower in farmers using carbamate in Kelantan and Selangor. This result supports findings by Wesseling et al. (2002), who reported that use of carbamates inhibits AChE activity. However, farmers using carbamate in Selangor had slightly higher AChE levels. Organochlorine pesticides (OC) are a class of chemicals widely in the 1940s shown to have negative effects on the environment. Although their use was banned in industrialized countries in the 1970s, they are still used in agriculture (Cruz et al. 2003) in some parts of the world, including locations such as Kelantan and Selangor.
OC reportedly has an affinity for α subunits of voltage-dependent sodium channels. This binding prevents closure of channel voltage, thus causing continuous impulse transmission (Karami Mohajeri and Abdollahi 2011). OC also has a neurogenic effect due to GABAA and glycine receptor inhibition (Heusinkveld and Westerink 2012). It was also found that OC act as an AChE activity inhibitor (Dutta and Arends, 2003). In this study, AChE levels in farmers using OC were significantly lower than those in farmers who did not use OC. This is likely because OC is a highly toxic pesticide (Salehhudin 1995) that causes AChE inhibition and continuous impulse transmission (Kamrin 1997). Ciesielski (1994) reported headache, blurred vision, muscle cramps, and lack of appetite to be positively correlated with AChE levels. These symptoms are also common manifestations of AChE inhibition (ATSDR 1993). The current study found that the symptoms associated with exposure to pesticides most commonly experienced by farmers were dizziness, headache, and cough. These findings support previous research by Yassin et al. (2002), who reported dizziness and headaches to be the most common symptoms associated with pesticide exposure.
AChE level was strongly negatively correlated with age and working period. Most farmers were between 41 and 64 years of age. This negative correlation suggests that AChE levels decrease with increasing age. Previous studies by Meena et al. (2009) also reported similar findings in observations and experiments conducted on mouse brains, with lower AChE levels in older mice compared to young mice.Among farmers in Kelantan and Selangor, 57% and 54%, respectively, had worked from six to 20 years. Farmers working for longer periods reportedly suffer health problems due to exposure to pesticides (WHO 1998). There is a strong correlation between working period and AChE levels. The findings of this study support those of Ohayo-Mitoko et al. (2000) in which AChE level were low even in farmers who had worked for less than 20 years.

CONCLUSION

AChE levels were low in the farmers in Kelantan and Selangor. The levels were lower in farmers using carbamates and organochlorines in Kelantan and higher in farmers using carbamates in Selangor. AChE levels were lower in farmers who reported headaches and dizziness. Increased age and working periods were associated with lower the AChE levels.

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REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1993). Case Studies In Environmental Medicine: Cholinesterase-Inhibiting Pesticide Toxicity. California: Department of Health and Human Services 3-8.

Assin MM, Abu Mourad TA, Safi JM (2002). Knowledge, Attitude, Practice, And Toxicity Symptoms Associated With Pesticide Use Among Farm Workers In The Gaza Strip. Occupational and environmental medicine59: 387-394.

Barry WW, Daniel EA, John DH (2005). Monitoring Cholinesterase To Detect Pesticide Exposure. Chemico-biological interactions 158: 253-256.

Ciesielski S, Loomis DP, Mims SR, Auer A (1994). Pesticide Exposures, Cholinesterase Depression, and Symptoms among North Carolina Migrant Farm workers. American Journal of Public Health 84: 446-451.

Cruz S,Lino C, Silveira MI (2003). Evaluation of organochlorine pesticide residues in human serum from an urban and two rural populations in Portugal. Science of the Total Environment 317: 23-25.

Cuna RHD, Vazqueza GR,Piolc MN, Guerreroc NV, Maggesea MC. (2011). Assessment ofthe Acute Toxicity ofthe Organochlorine Pesticide Endosulfan in Cichlasoma Dimerus (Teleostei, Perciformes. Ecotoxicology and Environmental Safety 74: 1065-1073.

Dhingra AK, Saini D, Chopra B, Parle M (2011). Psychopharmacological Investigation of the Nootropic Potential of Trigonella Foenum Linn in Mice. Asian Journal of Pharmaceutical and Clinical Research 4: 76-84.

Dutta HM, Arends DA (2003). Effects of Endosulfan on Brain Acetylcholinesterase Activity in Juvenile Bluegill Sunfish. Environmental research 91: 157-162.

Eckert S, Eyer P, Mückter H, Worek F (2006). Kinetic Analysis ofthe Protection Afforded By Reversible Inhibitors against Irreversible Inhibition of Acetylcholinesterase by Highly Toxic Organophosphorus Compounds. Biochemical pharmacology72: 344-357

Ellman GL,Courtney KD, Andres VJ, Featherstone RM (1961). A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochemical pharmacology7: 88-95.

Frederick MF (2009). Pesticide and Cholinesterase. Institute of Food and Agricultural Sciences. University of Florida.

Heusinkveld HJ, Westerink RH (2012). Organochlorine Insecticides Lindane and Dieldrin and their Binary Mixture Disturb Calcium Homeostasis in Dopaminergic PC12 Cells. Environmental science & technology46: 1842-1848.

Iorizzo L, Bianchi A, Gamberini G (1996). Assessment of Human Exposure to Pesticides in Greenhouses and Effectiveness of Personal Protective Devices. Archives of industrial hygiene and toxicology 4: 25-33.

Jamal HH, Noor HI, Syarif HL, Syed Mohd SA, Noraziah D (1995). Health Effects Related To Pesticide Use Among Paddy Farmers Of The Muda Area In Kedah, Malaysia.

Jamal HH, Noor Hassim I, Syarif L, Ariffin O, Noraziah D. (2000). Pesticide Exposure Symptoms and Serum Cholinesterase Activity among Malaysian Paddy Farmers. Buletin Kesihatan Masyarakat: 6

Kamrim MA (1997). Pesticide Profiles: Toxicity, Environmental Impact, And Fate. London: CRC Press.

Karami-Mohajeri S,Abdollahi M (2011). Toxic Influence of Organophosphate, Carbamate and Organochlorine Pesticides on Cellular Metabolism of Lipids, Proteins and Carbohydrates: A Systematic Review. Human & experimental toxicology 30: 1119-1140.

Kaufer D, Friedman A, Seidman S, Soreq H (1999). Anticholinesterases Induce Multigenic Transcriptional Feedback Response Suppressing Cholinergic Neurotransmission. Chemico-biological interactions 119: 349-60.

Kazi AI,Oommen A (2012). The Effect of Acute Severe Monocrotophos Poisoning on Inhibition, Expression and Activity of Acetylcholinesterase in Different Rat Brain Regions. Neurotoxicology33: 1284-1290.

Meena M,Pillai SR, Rahul BP (2009). Age Associated Alterations in Acetylcholinesterase and Modulatory Effect of BacopaMonienre(Linn) on Brain of Mice. Asian Journal of Microbiology, Biotechnology & Environmental Sciences Paper3: 563-567.

Ohayo-Mitoko GJ, Kromhout H,Karumba PN, Boleij JS (1999). Identification of Determinants Of Pesticide Exposure Among Kenyan Agricultural Workers Using Empirical Modelling. The Annals of occupational hygiene43: 519-525.

Ohayo-Mitoko GJ, Kromhout H, Simwa JM,Boleij JS, Heederik D. (2000). Self-Reported Symptoms and Inhibition of Acetylcholinesterase Activity among Kenyan Agricultural Workers. Occupational and environmental medicine57: 195-200.

Osten VEC, Tinoco-Ojanguren R,Soares AMVM, Guilhermino L (2004). Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. Archives of environmental health59: 418-425.

Reinecke SA, Reinecke AJ (2007). Biomarker Response and Biomass Chanfe of Earthworms Exposed To Chlorpyrifos in Microcosms. Ecotoxicology and Environmental Safety 66: 92-101.

Sallehudin S, Insektisid Dan KawalanVektorPembawaPenyakit. (1995). Dewan Bahasa & Pustaka, Kuala Lumpur.

Simonian AL, Good TA, Wang SS, Wild JR (2005). Nanoparticle-Based Optical Biosensors forthe Direct Detection of Organophosphate Chemical Warfare Agents and Pesticides. AnalyticaChimica Acta534: 69-77.

Suhail M, Rizvi SI (1989). Erythrocyte Membrane Acetylcholinesterase In Type 1 (Insulin-Dependent) Diabetes Mellitus. The Biochemical journal259: 897-899.

Ueyamaa J, Satoh T,Kondoa T,Takagia K,Shibatac E. (2009). B-Glucuronidase Activity is a Sensitive Biomarker to Assess Low-Level Organophosphorus Insecticide Exposure. Toxicology Letters193: 115-119.

Vidair CA (2004). Age Dependence of Organophosphate and Carbamate Neurotoxicity inthe Postnatal Rat: Extrapolation tothe Human. Toxicology and applied pharmacology196: 287-302.

Wesseling C, Keifer M, Ahlbom A, McConnell R, Moon J. (2002). Long-Term Neurobehavioral Effects Of Mild Poisonings With Organophosphate and Nmethyl Carbamate Pesticides among Banana Workers. International journal of occupational and environmental health8: 27-34.

World Health Organization (WHO) Chemical Safety. (1998). 51th Year, World Health Organization 28: 9.

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