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Article *in* Indian Journal of Forensic Medicine & Toxicology · October 2020

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Abstract

PON1 Q192R (rs662) polymorphism in coding region is conveying differential catalytic activity of organophosphate. Study was aimed to investigate PON1 Q192R gene polymorphisms in rice farmers along with pesticide knowledge and practices; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. Information of pesticide exposure, knowledge and practices were collected from 50 rice farmers and 50 control respondents by questionnaire interviewing. Each blood sample was obtained by venipuncture and prepared to serum for SChE activity test by paper test and automatic analyzer; to EDTA blood for genotyping by PCR-RFLP. Exposure status, knowledge and practices on pesticide use were represented as frequency. Chi-square was used to analyze on the different of personal information; and of polymorphism. Independent *t*-test was used for comparison of SChE levels. Person correlation was evaluated relationship between SChE level and genotypes. 60% of rice farmers were used pesticide over 10 years. Rice farmers were long-term pesticide exposure with good on pesticide knowledge, however poorly practice. Means of SChE level of both groups were within reference value and significantly different (p = 0.033). Polymorphisms were included wild type (QQ), heterozygote (QR) and homozygote (RR); and were significantly different between rice farmers (high frequency of RR genotype) and control (p = 0.02). Relationship of polymorphism and SChE level were negative correlated (r = -0.261; p = 0.031). This polymorphism may useful biomarker for chronic pesticide exposure in Thai rice farmer and corresponded to decline of serum cholinesterase; and RR genotype was high risk group.

Keywords: *chronic pesticide exposure, paraoxonase-1 (PON1), PON1 Q192R polymorphism, serum cholinesterase*

Introduction

Many previous studies were reviewed regarding pesticide use, poisoning, and knowledge and unsafe occupational practices in Thailand over the last decade; and Thai government is responsible in making policies and regulations and encouraging all agricultural activities to be sustainable [1]. Rice farmers in three Thai provinces were found to have the high prevalence of allergies, nasal congestion, wheezing, and acute symptoms after pesticide use [2]. Occupational health and safety problems among rice farmers may result from unsafe behaviors and unsafe acts or practices of workers, which are related to agrochemical exposure, such as the use of faulty spraying equipment or lack of attention to safety precautions [3]. Suphan Buri province

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is located in central river plain of Thailand. Because of enough water supplies in this cultivation area, two or three rice growing cycles can be done. Thus, farmers may expose to pesticides more frequent rather than other area [4]. Moreover, outsource pesticide sprayers were common finding in rice field. Chloropyrifos and carbofuran are commonly used in this area, which are the member of organophosphate (OP) and carbamates (CB), respectively [1, 5]. Decline of serum cholinesterase is useful to indicate risk of pesticide exposure especially in sprayers [6-7]. Thai government is developing cholinesterase activity screening test and national apply for control the risk and unsafe field workers [8].

Paraoxonase 1 (PON1) is a phase-I enzyme that is involved in the hydrolysis of organophosphate esters [9, 10]. Literature reviews of PON1 genotypes are involving in the presence of Parkinson’s disease, Alzheimer’s disease and amyotrophic lateral sclerosis [11]. PON1 Q192R genotype is also associated with the risk of multiple cancers [12, 13] and coronary heart diseases [14]. PON1 variants play a role in xenobiotic-metabolizing system in occupational exposures and consequence to oxidative stress and DNA damage [15]. Human PON1 Q192R (rs662) polymorphism in coding region is conveying differential catalytic activity toward some OP substrates [16]. PON1 Q192R polymorphism (rs662A > G) was caused by the glutamine (Q genotype) substituted for the arginine (R genotype) 192 of the gene 6 exon of the PON 1 gene [17]. The information of PON1 Q192R gene polymorphisms among Thai race especially agricultural workers was still limited. This study was aimed to investigate PON1 Q192R gene polymorphisms in rice farmers along with pesticide knowledge and practices; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels.

Materials and Methods

Subject recruitment and data collection

The cross-sectional study was carried out from June 2019 to February 2020 on data had collected from annually health service program by health promoting hospital, This study was recruited 100 respondents included 1) 50 rice farmers (risk group) were aged 18-65 yr who lived in this area, which had handle pesticide regularly or work in paddy field at least three years or more 2) The control group was included 50 respondents who lived nearby field area and listed in house registration, had non-related professional for farm workers. Respondents with a history of serious conditions were excluded. Questionnaire interviewing and blood collection were conducted by well-trained research assistants and medical technologists, respectively. Gathered information concerning of long-term pesticide exposures including personnel information, adverse health symptoms, knowledge and practice of pesticide use were recorded from questionnaires by personnel interviewing. The sample size was estimated using the single proportion formula with 95% confidence interval and based on percentage of abnormal SChE level in previous study [18]. The Ethics Committee of Thammasat University was approved this research protocol (COA No. 084/2562). The director of U-Thong district ¢s health promoting hospital, Suphan Buri province gave permission to conduct on this study. All participants gave informed consented.

Blood collection, preparation and storage

Each 5 ml of blood sample was obtained by venepuncture from median cubital vein during morning (7-9 a.m.); and drawn into clotting blood and EDTA tubes for 3 ml and 2 ml, respectively. Clotting blood tube was further centrifuged; and serum was separated within 2 h after phlebotomy and stored at -20 °C [19] for SChE activity test. Whole blood contained in EDTA tubes were prepared for genomic DNA extraction by using the QIAamp blood DNA mini kit (QIAGEN Thailand, Bangkok, Thailand) and genomic DNA was stored at -20 °C.

SChE activity test

Screening of SChE level by paper test [8] and confirming of SChE level was done by automatic analyser. The paper test kit was developed and manufactured by Government Pharmaceutical Organization (GPO), Thailand. The efficiency of test including sensitivity, specificity and positive predictive values were 77, 90 and 85%, respectively. The quantitative for SChE level was conducted by automatic analyzer, COBAS c501 (Roche-diagnostics, Rotkreuz, Switzerland), which were performed in certified clinical laboratories. SChE level were interpreted by reference values according by instruction of manufacturer.

PON1 Q192R polymorphisms by PCR-RFLP

DNA template was amplified by polymerase chain reaction (PCR) using forward 5′-TAT TGT TGC TGT GGG ACC TGA G-3′ and reverse 5′-CCT GAG AAT CTG AGT AAA TCC ACT-3′ primers, which were corresponded to PON1(Q192R) region [20]. PCR was performed in 25 µl of total volume, which was contained 2 µl of genomic DNA, 0.5 µl of each primer, 5 µl of 10X PCR buffer (1.5 mM Mg^{2+}) include dNTP mixture and 0.25 µl of 1.5 U AmpliTaq polymerase (Thermo Fisher Scientific, USA). PCR was performed with initial denaturation at 94°C for 5 min. followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72 °C for 30 s followed by final extension at 72 °C for 7 min Thermal cycler (Applied Biosystems, USA). Restriction fragment length polymorphism (RFLP) was performed: DNA product was digested by *A1wI* restriction enzyme (New England Bio Labs, Cambridge, UK). Digested DNA fragments was separated on 2% of agarose gel electrophoresis apparatus then stained with ethidium bromide. DNA electrophorogram was read by using ultraviolet transillumination (Promega, USA). PON1 Q192R single nucleotide polymorphisms (SNPs) were 1) 66- and 172-bp fragments for the 192R allele and 2) 238-bp fragment (undigested) for the 192Q allele. The interpretation of PON1 Q192R SNPs on genotypes were represented 238-bp fragment for wild type (QQ); 66, 172, 238-bp fragments for heterozygous (QR); and 66, 172-bp fragments for homozygous (RR). Quality control of test was done by DNA sequencing, which was randomized from 15% of samples.

Statistical Analysis

Descriptive data was explained by using frequency. Chi-square was used to analyze on the different of personal information and genotypes between rice farmer and controls groups. Independent *t*-test was used for comparison of SChE level between rice farmer and controls groups; and between wild and variant types. Person correlation was evaluated between SChE level and genotypes. The statistical significance was judged at $p< 0.05$. SPSS 21.0 software was used for statistical analysis (SPSS, Chicago, Illinois, USA).

Results and Discussion

Pesticide exposure, knowledge and practice of rice farmers

All of rice farmers were long-term pesticide exposure and 60% of rice farmers were used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. The related pesticide used symptoms were rarely occurred. Unexpected finding may due to unspecific symptoms, imprecisely explain by personal interviewing and tolerance of frequent exposed farmers. Means of SChE level were significantly different between rice farmers and controls ($p = 0.033$), however, there were within reference value (Table 1). Most of rice farmers were known about pesticide on health effects (80%), however, some of them were improper on practice, such as, the reading of pesticide label, first aids for toxicity and appropriated skill on spraying (Table 2). Educational interventions are essential for promoting safety during all phases of pesticide handling in small-scale Thai farmers. Public policies should be developed to encourage farmers to change their pest management methods from chemical based to methods that are healthier and more environmentally friendly [21]. Promotion of health literacy is associated to reduce unsafe behaviors on pesticide use [22].

PON1 Q192R polymorphisms in rice farmers

The DNA electrophoresis of PON1 Q192R gene polymorphisms were included wild type (QQ), heterozygote (QR) and homozygote (RR) (Fig. 1). The genotypic polymorphisms of rice farmers and control were significantly different (p=0.02); rice farmers were more frequent RR genotype rather than control (Table 3). The relationship of PON1 Q192R gene and SChE level were negative correlated ($r = -0.261$) with statistically significant ($p = 0.031$) (Table 4). Six of rice farmers were low SChE level (<5,500 U/L) and RR genotype polymorphism (data not show).

In this study, SChE and PON1 Q192R genotype between rice farmers and controls were significantly different; and the decline of SChE and PON1 Q192R genotype were also related. The significant finding was corresponded to previous study, which was reported that PON1 192RR genotype and CYP2D6 1934A allele are relate to organophosphate susceptibility in chronic exposure in Egyptians. Moreover, SChE is significantly reduced in chronic organophosphate-intoxicated Egyptian patients [23]. PON1 192 R(+) (QR + RR genotypes) genotype carriers had higher PON1 and acethylcholinesterase (AChE) activities than 192 R(-) (QQ) genotype carriers in Turkish population, which had chronic pesticide exposure in occupational reasons [24]. In our study, rice farmers and control were significantly different and rice farmers were older than control. Q192R and L55M polymorphisms of PON1 are population-specific effects due to interaction of gene variation and environmental factors; therefore, this polymorphism is not impact on elders or extreme ages [25]. The most of pesticide exposure is chronic rather than acute toxicity via occupational and/or environmental means. Pesticide may lead harmful effects of human health, which are through oxidative stress, epigenetic transformation, and gene polymorphisms. Most of gene polymorphisms are implicate to OP metabolism enzymes, such as cytochrome P450, glutathione transferase, acetyltransferases or paraoxonase [26]. R variant of PON1 Q192R gene polymorphism is less effective to protect LDL oxidation. Thus, R genotype is associate to hypertension, coronary artery diseases, stroke and Parkinson's disease [27, 28]. Large scale and seasonal variation studies on gene polymorphism and environmental expose will conduct to confirming the finding.

Table 1: The frequency of personal data and pesticide-exposing factors from rice farmers and controls

Personal data/ Exposing factors	Rice farmer (%)	Control (%)	p-value
Gender : Male	34 (68)	30 (60)	0.405
Female	16 (32)	20 (40)	
Age : < 40 years	22 (44)	40 (80)	0.0001*
≥ 40 years	28 (56)	10 (20)	
Alcohol intake: none	36 (72)	33 (66)	0.517
drinking	14 (28)	17 (34)	
Duration of pesticide use: 4-9 years	18 (36)	-	
> 10 years	32 (64)	-	
Frequency of exposure: 1-2 days/week	30 (60)	-	
3-4 days/week	4 (8)	-	
5-6 days/week	16 (32)	-	
Clinical symptoms: None	36 (72)	-	
Headache/vertigo	11 (22)	-	
Abdominal cramp	3 (6)	-	
Health education: none	13 (26)	-	
educated	37 (74)	-	
Serum cholinesterase (U/L) **	7247.2 ± 1293.3	7775.9 ± 1152.8	0.033*

* Statistically significant at $p<0.05$; ** Serum cholinesterase (SChE) was screened by paper test before tested with automatic analyzer and represented as mean ±SD (reference value = 5,500-13,000 U/L).

Table 2: Knowledge and practice of rice farmers for pesticide use

Topic of Pesticide use	Frequency (%)	
	Uneducated	Educated
Health effects of pesticide	10 (20)	40 (80)
First-aids for acute intoxication	23 (46)	27 (54)
Personal protective equipment	17 (34)	33 (66)
Reading and understanding on information of pesticide label	24 (48)	26 (52)
Cleaning of pesticide spraying clothes	15 (30)	35 (70)
Pesticide container management	17 (34)	33 (66)
Appropriate in pesticide spraying	21 (42)	29 (58)
Personal hygiene after pesticide spraying	15 (30)	35 (70)

Table 3: Genotypic frequency of PON1 Q192R in rice farmer and control groups

Genotype	Control		Farmer		p-value
	N	%	N	%	
Wild Type (QQ genotype)	29	58	14	28	0.020*
Polymorphisms (RR & QR genotype)	21	40	36	72	
Homozygous (RR genotype)	12	24	27	54	
Heterozygous (QR genotype)	9	18	9	18	

* Statistically significant at $p<0.05$

Table 4: Relationship of PON1 Q192R genotypes and SChE level

Genotype	N	SChE (U/L)	r	p-value
Wild Type	43	7820.14 ± 1103.25	-0.261	0.031*
Polymorphisms	57	7278.89 ± 1307.70		

* Statistically significant at $p<0.05$; r = Pearson correlation coefficient

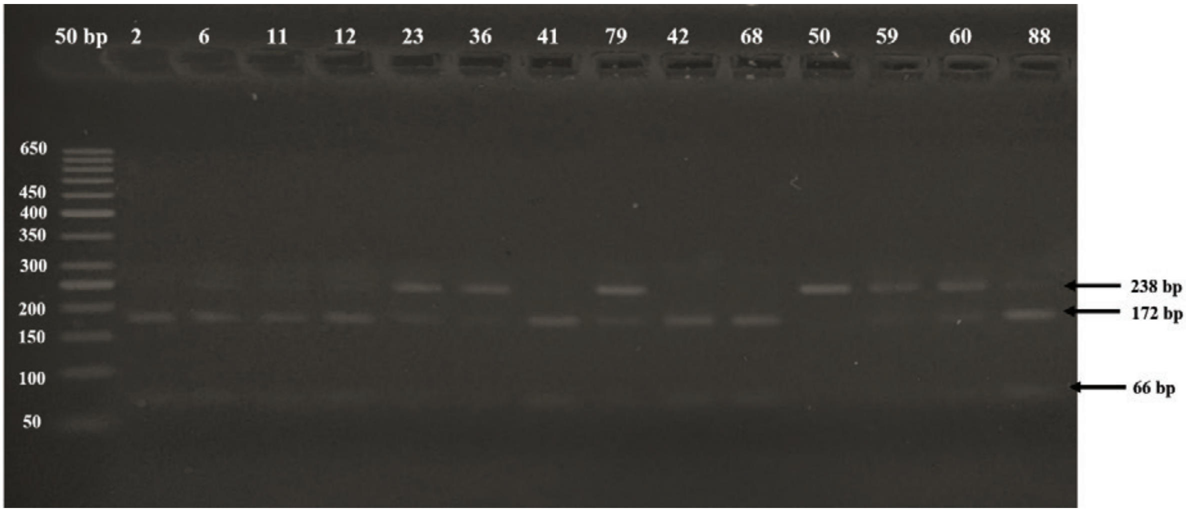

Figure 1: DNA fragment separation. First Lane (from left): DNA ladder; Lane 2, 6, 11, 12, 41, 42, 68 and 88: 66, 172-bp for RR genotype; Lane 23, 36 and 79: 66, 172, 238-bp for QR genotype; Lane 23, 36 and 79: 238-bp for wild type (QQ genotype).

Conclusion

PON1 Q192R polymorphism may useful biomarker for chronic pesticide exposure in Thai rice farmer and corresponded to decline of SChE; and RR genotype was high risk group.

Acknowledgement: We would like to sincerely thank medical staffs from U-Thong district ¢s health promoting hospital for local public relation and research assistants providing.

Source of Funding: Thammasat University and Suan Sunandha Rajabhat University, Thailand.

Ethical Clearance: Ethics Committee of Thammasat University was approved this research protocol (COA No. 084/2562).

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

References

1. Sapbamrer R. Pesticide use, poisoning, and knowledge and unsafe occupational practices in Thailand. New Solutions. 2018; 28(2): 283-302.
2. Kongtip P, Nankongnab N, Mahaboonpeeti R, Bootsikeaw S, Batsungnoen K, Hanchenlaksh C, Tipayamongkholgul M, et al. Differences among Thai Agricultural Workers' Health, Working Conditions, and Pesticide Use by Farm Type. Ann Work Expo Health. 2018; 62(2): 167-181.
3. Buranatrevedh S, Sweatsriskul P. Model development for health promotion and control of agricultural occupational health hazards and accidents in Pathumthani, Thailand. Ind Health. 2005; 43(4): 669-676.
4. Grandstaff S, Srisupun W. Agropesticide contract sprayers in Central Thailand: Health risks and awareness. Southeast Asian Stud. 2004; 42(2): 111-131.
5. Prasertsung N. Situation of pesticides used in rice fields in Suphanburi Province; Proceedings of the conference on chemical pesticides. Nov 15-16, 2012. Available from: www.thaipan.org/sites/default/files/conference2555/conference2555_1_10.pdf.
6. Giacobini, E. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol Res. 2004; 50: 433-440.
7. Sudjaroen, Y, Suwannahong, K. Comparison of Biochemical, Hematological Parameters and Pesticide Expose-related Symptoms among Organic and Non-organic Farmers, Singburi, Thailand. Asian J Pharm. 2017; 11: S235-S241.
8. Bureau of Policy and Strategy, Ministry of Public Health. Thailand health profile report 2005-2007,

Bangkok: The War Veterans Organization of Thailand, 2008.

9. Costa LG, Richter RJ, Li WF, Cole T, Guizzetti M. Paraoxonase (PON1) as a biomarker of susceptibility for organophosphate toxicity. Biomarkers. 2003; 8: 1-12.
10. Ellison CA, Crane AL, Bonner MR, Knaak JB, Browne RW, Lein PJ, Olson JR. PON1 status does not influence cholinesterase activity in Egyptian agricultural workers exposed to chlorpyrifos. Toxicol Appl Pharmacol. 2012; 265: 308-315.
11. Androutsopoulos VP, Kanavouras K, Tsatsakis AM. Role of paraoxonase 1 (PON1) in organophosphate metabolism: implications in neurodegenerative diseases. Toxicol Appl Pharmacol. 2011; 256: 418-424.
12. Liu P, Wang Q, Cui Y, Wang J. A meta-analysis of the relationship between paraoxonase 1 polymorphisms and cancer. Free Radic Res. 2019; 53(11-12): 1045-1050.
13. Pan X, Huang L, Li M, Mo D, Liang Y, Liu Z, Huang Z, Huang L, Liu J, Zhu B. The Association between PON1 (Q192R and L55M) gene polymorphisms and risk of cancer: a meta-analysis based on 43 studies. Biomed Res Int. 2019; 2019: 5897505.
14. Amini M, Esmaeilzadeh-Bahabadi S, Avan A, Gholoobi A, Ghasemi F, Mirhafez SR, et al. Paraoxonase-1 Q192R polymorphism and its association with hs-CRP and fasting blood glucose levels and risk of coronary artery disease. Diabetes Metab Syndr. 2019; 13(2): 1053-1057.
15. Kahl VFS, da Silva FR, Alves JDS, da Silva GF, Picinini J, Dhillon VS, et al. Role of PON1, SOD2, OGG1, XRCC1, and XRCC4 polymorphisms on modulation of DNA damage in workers occupationally exposed to pesticides. Ecotoxicol Environ Saf. 2018; 159: 164-171.
16. Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE. Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. Toxicology. 2013; 307: 115-122.
17. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet. 2001;68(6):1428–1436.
18. Kachaiyaphum P, Howteerakul N, Sujirarat D, Siri S, Suwannapong N. Serum cholinesterase levels of Thai chili-farm workers exposed to chemical pesticides: prevalence estimates and associated factors. J Occup Health. 2010; 52: 89-98.
19. Young DS, Bermes EW. Specimen collection and processing: Sources of biological variation. In: Burtis CA, Ashwood AR, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. 1999. Philadelphia: Saunders, pp. 42-72.
20. Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Fontana L, Castaldo M, et al. Association between serum paraoxonase (PON1) gene promoter T(-107) C polymorphism, PON1 activity and HDL levels in healthy Sicilian octogenarians. Exp Gerontol. 2004; 39(7): 1089-1094.
21. Plianbangchang P, Jetiyanon K, Wittaya-Areekul S. Pesticide use patterns among small-scale farmers: a case study from Phitsanulok, Thailand. Southeast Asian J Trop Med Public Health. 2009; 40(2): 401-410.
22. Pobhirun T, Pinitsoontorn S. The association between health literacy and pesticide use behaviors among sweet corn farmers in the Pak Chong district of Thailand: a cross-sectional study. F1000Res. 2019; 8: 448.
23. Tawfik Khattab AM, Zayed AA, Ahmed AI, Abdel Aal AG, Mekdad AA. The role of PON1 and CYP2D6 genes in susceptibility to organophosphorus chronic intoxication in Egyptian patients. Neurotoxicology. 2016; 53: 102-107.
24. Sunay SZ, Kayaaltı Z, Bayrak T, Söylemezoğlu T. Effect of paraoxonase-1 192 Q/R polymorphism on paraoxonase and acetylcholinesterase enzyme activities in a Turkish population exposed to organophosphate. Toxicol Ind Health. 2015; 31: 1061-1068.
25. Wei GZ, Zhu MY, Wang F, Zhao YG, Li SS, Liu TY, *et al.* Paraoxonase (PON1) polymorphisms Q192R and L55M are not associated with human longevity: A meta-analysis. Z Gerontol Geriatr. 2016; 49: 24-31.

26. Teodoro M, Briguglio G, Fenga C, Costa C. Genetic polymorphisms as determinants of pesticide toxicity: Recent advances. Toxicol Rep. 2019; 6: 564-570.

27. Rohr P, da Silva PJ, Erdtmann B, Saffi J, Guecheva TN, Antônio Pêgas Henriques J, *et al.* BER gene polymorphisms (OGG1 Ser326Cys and XRCC1 Arg194Trp) and modulation of DNA damage due to pesticides exposure, Environ Mol Mutagen. 2011; 52: 20-27.

28. Menini T, Gugliucci A. Paraoxonase 1 in neurological disorders. Redox Rep. 2014; 19: 49-58.