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Research Article

Association of pon1 q192r gene and metabolic disorder in chronic pesticide exposures

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ABSTRACT

Paraoxonase 1 (PON1) enzyme is involved in organophosphate metabolism and neurodegenerative disorders, which is biomarker for dyslipidaemia, insulin resistance, cardiovascular diseases and hypothyroidism. Aims of this study were to investigate PON1 Q192R (rs662) gene polymorphism; and to evaluate the association of genotype and metabolic biomarkers in chronic pesticide exposures. Information concerning of pesticide exposures from 50 rice farmers and 50 control respondents was collected by questionnaire interviewing. Blood sample (6 ml) was collected from each volunteer and separated to serum, NaF- and EDTA-plasma. Serum cholinesterase (SChE), liver function test (LFT), fasting blood glucose (FBG), HbA1c and lipid profile was assessed using automatic analysers; and PON1 Q192R genotyping was analysed by PCR-RFLP. All of rice farmers were chronic exposed and more than 50% of them was used pesticide over ten years. Average of SChE level of both groups were significantly different, however, there were within normal range. Genotypes were significantly different between rice farmers (high frequency of RR genotype) and control (p=0.02). Metabolic parameters including FBG, HbA1c, cholesterol, triglyceride, HDL-c and LDL-c in rice farmers and controls were different (p = 0.035, 0.045, 0.03, 0.021, 0.037 and 0.025, respectively). RR genotype was associated to metabolic disorders including insulin resistance and dyslipidaemia in chronic pesticide exposures. This genotypic variation was need to be consider on metabolic disorder treatment especially statins therapy.

Keywords: cholinesterase inhibitors, dyslipidaemia, insulin resistance, metabolic disorder, paraoxonase-1 (PON1), PON1 Q192R genotype

INTRODUCTION

Various effects of chronic pesticide exposure are cancer, birth defects, reproductive disorders, neurodegenerative, cardiovascular and respiratory diseases, developmental disorders, metabolic disorders, chronic renal disorders or autoimmune diseases (Czajka et al., 2019). According epidemiologic data, pesticides are effect on enzymes, which are responsible for liver function; blood cell characteristics and other biochemical pathways in persons who are pesticide expose and have occupationally diseases (García-García et al., 2016; Pothu et al., 2019; Kori et al., 2019). Effects of pesticide intoxication are depending on free radical production, environmental factors, physiological transformation and genotypic variation (Karami-Mohajeri et al., 2011; Teodoro et al., 2019). Light occupational exposure to pesticides is associated with haematological abnormalities. However, prolong and intensive exposure may associate to any liver, kidney, or haematological disorders (Neghab et al., 2018). Organophosphate (OP) and carbamates (CB) are act as acetyl cholinesterase (AChE) inhibitors and affect to several organs, such as peripheral and central nervous systems, muscles, liver and pancreas. Impairment of enzymatic pathways modulated biochemical metabolism within cytosol, mitochondria, and peroxisomes may cause by AChE inhibition at target organs (Pohanka, 2019; King and Aaron, 2015; Karami-Mohajeri and Abdollahi, 2011). Sub-chronic exposure to organophosphate can causes hyperlipidaemia, reduced glycogen storage in the liver, and increased oxidative stress, which is associated with the risk for many chronic diseases, such as cardiovascular diseases, hypertension, and diabetes mellitus in animal models (Acker and Nogueira, 2012; Elsharkawy

et al., 2013); and in occupational exposures (Patil et al., 2009; Sudjaroen, 2015; Sudjaroen and Suwannahong, 2017).

Paraoxonase 1 (PON1) is a phase-I enzyme that is involved in the hydrolysis of organophosphate esters (Costa et al., 2003; Ellison et al., 2012). Literature reviews of PON1 genotypes are involving in the presence of Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis (Androutsopoulos et al., 2011; Menini and Gugliucci, 2014). PON-1 is also biomarker for dyslipidaemia, insulin resistance, cardiovascular diseases and hypothyroidism (Sampson et al., 2005; Siewert et al., 2015; Singh and Dey Sarkar, 2014). Human PON1 Q192R (rs662) polymorphism in coding region is conveying differential catalytic activity toward organophosphate (Costa et al., 2013); and is associated to metabolic disorder and cardiovascular disease (Alegría-Torres et al., 2015; Srinivasan et al., 2004; Rodríguez-Carrio et al., 2016). Aims of this study were to investigate PON1 Q192R gene polymorphisms in chronic pesticide exposures along with occupational health risks and behaviours; and to evaluate the association of PON1 Q192R gene polymorphism and biochemical parameters, including serum cholinesterase (SChE), fasting blood glucose (FBG), HbA1c, lipid profile and liver function test. The finding may represent interaction of genetic variation and metabolic disturbance by xenobiotic effect (such as pesticide) on Thai chronic exposures. Further study can be conduct on other xenobiotic in pharmacological or toxicological aspects.

SUBJECTS AND PROTOCOLS

Study area and pesticide use

U-Thong district, Suphan Buri is locate about 150 km from Western of Bangkok. Various rice varieties are originating and cultivate in this area. Growing cycle of rice is two to three times per years, because enough water supply. Chlorpyrifos, carbofuran, paraquat and glyphosate are most favour uses. Outsource pesticide sprayers were regularly finding in rice field workers.

Subjects and questionnaire interviewing

Cross-sectional study was conducted on routine health service program during July 2019 until January 2020. One hundred of subjects were recruited and divided to rice farmer and control groups (N = 50 for each). Inclusion criteria of rice farmer group were 18-65-year-old who resided within study area and frequently work directly or related with pesticide in paddy field at least 3 years ago. The control group was respondents who resided nearby agricultural area, each name included in census of study area, and occupational status was not related to agricultural workers. Subjects who concerned with history of severe state of chronic diseases, such as liver diseases, kidney diseases, cardiovascular diseases and cancer were excluded from this study. Personal data was collected from subjects by research assistants, and venipuncture for blood collection was done by senior medical technologist. Data of chronic pesticide exposure was consisted of adverse relate-symptoms, history of personal protective equipment (PPE) use, and good practice of pesticide use were recorded by interviewing. The estimation of sample size was calculated from formula and frequency of abnormal SChE farmers in previous study (Kachaiyaphum et al., 2010). This research protocol had approved from Ethical Committee of Thammasat University, Thailand (COA No. 048/2562). Health promoting hospital, which responsible to health service at U-Thong district area, was allowed to carry on this study.

Sample collection and preparation

Each blood sample (6 ml) was collected from median cubital vein of each subject by venepuncture at early morning, which was separated to plain, NaF and EDTA tubes for 3, 1.5 and 1.5 ml, respectively. Plain tube was clotted and then centrifuged within 2 h for serum separation. NaF tube and EDTA tubes were prepared NaF plasma and EDTA whole blood (Young and Bermes, 1999). Serum, NaF plasma and EDTA whole blood were used to determine for biochemical, FBG and HbA1c tests, respectively. The remaining of EDTA whole blood was extracted for DNA template by QIAamp blood DNA mini kit (QIAGEN Thailand, Bangkok, Thailand); and genomic DNA was stored at -20 °C.

DETERMINATION OF BIOCHEMICAL PARAMETERS

The determination of SChE, LFT, lipid profile and FBG was analysed by COBAS c501 (Roche-diagnostics, Rotkreuz, Switzerland). Percentage of HbA1c were determined by Celltac E MEK-7222 (Nihon Kohden, Tomioka, Japan). All of laboratory tests were run by certified standard procedures. Laboratory interpretation was decided according by manufacturer 's instruction and clinical and laboratory standards institute (CLSI).

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 represented to PON1(Q192R) region (Campo et al., 2004). PCR was performed in 25 μl of total mixture, 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 and 0.25 μl of 1.5 U AmpliTaq polymerase (Thermo Fisher Scientific, USA). Each cycle was programed with initial denaturation at 94°C for 5 min. followed by 35 cycles comprising 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: A1wl restriction enzyme (New England Bio Labs, Cambridge, UK) was used to hydrolyse PCR product. 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 polymorphisms 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). DNA sequencing was confirmed on 15% of randomizing samples as quality assurance on this genotypic study.

Statistical Analysis

Kolmogorov-Smirnov test was performed on analysis of data distribution. Descriptive statistic was reported by percentage, mean and standard deviation. Different of personal data and PON1 Q192R polymorphisms between rice farmers and controls were analysed by Chi-square. Different of biochemical parameters between two groups and between wild type and variant were analysed by independent t-test. Statistical analysis was performed by SPSS 21.0 program (SPSS, Chicago, Illinois, USA) and statistical significance was judged at $p < 0.05$.

RESULTS AND DISCUSSION

PESTICIDE EXPOSURE STATUS IN RICE FARMERS

The personal data of two group, such as gender and alcoholic consumption was not different; and majority of farmers were men and more than forty years old. All of rice farmers were chronic exposed and more than 50% of them was used pesticide over ten years.; and majority of frequency was 1-2 time/week. The related pesticide used symptoms were unusually appeared and mainly of farmers had health education for consciousness of pesticide contact and can be protecting themselves. Average of SChE level of both groups were significantly different ($p = 0.033$), however, there were within normal range (Table 1). By our results, rice farmers were chronic pesticide exposure and frequently contacted with pesticide. Therefore, appearance of related symptoms was rarely, non-specific and difficult to defining. Unanticipated finding has affected by imprecisely explanation of personal interviewing and physiological tolerance of frequent exposure. Rice farmers were mainly understood on health awareness and protection from pesticide use.

Only six of rice farmers had low SChE level (<5,500 U/L; data not show), which was implied that serum cholinesterase may not be a good marker for quantifying exposure to pesticide among sprayers, especially during spraying season. In previous study, agriculturists can be exposed to pesticides divided into sprayers, agriculturists and other professions, however, the SChE levels among them were not significantly difference and level was still within reference range (Mathew et al., 2015). Many reports of pesticide exposure in Thai farmers were provides single type of pesticide exposed; therefore, mixed pesticide uses are more common for multi-crop cultivation. Hence, evaluation of chronic pesticide exposure is further interesting on other biomarker determination rather SChE, such as alkyl phosphate metabolites (DAPs), urinary 3-phenoxybenzoic acid (3-PBA) and urinary glyphosate (Wongta et al., 2018).

GENE POLYMORPHISMS AND METABOLIC DISTURBANCE IN CHRONIC EXPOSURE

The DNA electrophoresis of PON1 Q192R gene polymorphisms were represented to wild-type (QQ), heterozygote (QR) and homozygote (RR) (Fig. 1). PON1 Q192R gene polymorphisms of rice farmers and control were significantly different ($p=0.02$); and more frequent RR genotype (Table 2). Moreover, six of rice farmers were homozygous polymorphism (RR genotype) and abnormal SChE level. Our significant finding was corresponded to previous study, which was reported that PON1 192RR genotype allele is relate to organophosphate intoxication in chronic exposure in Egyptians. Moreover, the reduction of SChE had reported in chronic organophosphate-intoxicated Egyptian patients (Tawfik Khattab et al., 2016). 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 (Sunay et al., 2015). In our study, rice farmers and control were significantly

different and age of rice farmers were older. 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 (Wei et al., 2016).

LFT level of two groups was not different, and there were within normal values (Table 3). However, metabolic statuses were significantly difference between two groups; and p-value of FBG, HbA1c, cholesterol, triglyceride, HDL-c and LDL-c were 0.035, 0.045, 0.03, 0.021, 0.037 and 0.025, respectively (Table 4). Hence, rice farmers seemed to metabolic disorder including insulin resistance and dyslipidemia. 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 (Rohr et al., 2011; Menini and Gugliucci, 2014). Statins are affecting to PON-1 on improvement of antioxidant properties and insulin secretion; and glucose-lowering effects of statins are most pronounced in patients with an improvement in HDL-C upon statin therapy (Ferretti et al., 2015; Koren-Gluzer et al., 2011). The effect of statins is depending on PON-1 genotype especially Q192R polymorphism. Treatment of statins in Q allele patients can improve glucose metabolism, especially in insulin secretion (de Souza et al., 2015; Sumi et al., 2017). Corresponding to our results, RR genotype in chronic exposure might be risk for insulin resistance, dyslipidaemia and cardiovascular disease. This finding is suggest that xenobiotic from environment, such as pesticide exposure may "overlap" to metabolic markers and should be consider on clinical history for metabolic disorder treatment especially in statins or other relating drug therapy.

CONCLUSION

RR genotype of PON-1 Q192R polymorphism in chronic pesticide exposures was associated to metabolic disorders including insulin resistance and dyslipidaemia. This genotypic variation was need to be consider on metabolic disorder treatment especially statins therapy.

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Conflict of interest: The authors were confirmed that conflicts of interest were none.

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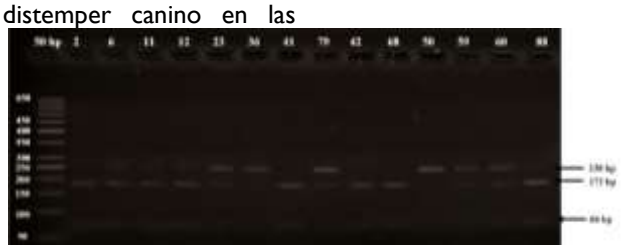

Fig.1: DNA electrophoresis separation of PON1 (Q192R) gene (rs662): First Lane (left hand side): DNA ladder 50-650 bp; Lane 2, 6, 11, 12, 41, 42, 68 and 88: DNA fragments (66, 172-bp) for homozygous genotype (RR); Lane 23, 36 and 79: DNA fragments (66, 172, 238-bp) for

heterozygous genotype (QR); and Lane 23, 36 and 79: DNA fragments (238-bp) for wild type genotype (RR).

Table 1: The personal data and risk of pesticide exposure from rice farmers

Personal data/ Risk	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*

* Significant at p<0.05; ** Serum cholinesterase (SChE) was preliminary screened by paper test (Semi-quantitative) and confirmed for SChE level by automatic analyzer (normal range = 5,500-13,000 U/L).

Table 2: 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 3: Liver function test in rice farmers and controls

Group/ parameter	Liver function test						
	Total protein (g/dL)	Albumin (g/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Rice farmer	7.29 ± 0.22	4.50 ± 0.04	0.36 ± 0.02	0.13 ± 0.03	23.0 ± 1.2	21.7 ± 2.0	99.9 ± 3.5
Control	7.45 ± 0.16	4.43 ± 0.04	0.36 ± 0.04	0.11 ± 0.01	23.6 ± 1.3	24.6 ± 1.9	101.2 ± 4.0
Reference range	6.60-8.70	3.50-5.50	0.30-1.20	0.00-0.50	0-37.0	0-40.0	53-128
p-value	0.552	0.282	0.964	0.485	0.744	0.308	0.797

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Table 4: FBG, HbA1c and lipid profiles in rice farmers and controls

Group/ parameter	FBG (mg/dL)	HbA1c (%)	Lipid profiles			
			Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
Rice farmer	109.5 ± 11.4	5.9 ± 0.8	208.5 ± 19.6	195.0 ± 17.3	48.1 ± 3.3	141.2 ± 31.5
Control	95.1 ± 12.5	4.6 ± 1.2	182.9 ± 15.3	163.2 ± 13.1	35.3 ± 4.7	123.1 ± 25.2
Reference range	70-110	< 6.5	0-200	50-200	35-100	70-160
p-value	0.035*	0.045*	0.03*	0.021*	0.037*	0.025*

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