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

CYP2D6 (G1934A) Gene Polymorphism in Rice Farmers with Long-term Pesticide Exposure, Suphan Buri, Thailand

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Abstract

CYP2D6 G1934A (rs 3892097) gene polymorphism in coding region is leads to catalytic rate of cytochrome P450 enzyme activity toward xenobiotic, including insecticide. Study was aimed to investigate CYP2D6 (G1934A) gene polymorphism in rice farmers along with occupational health risks and behaviors; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. Cross-sectional study was carried out from June 2019 to February 2020. Data had collected from health service program by U-Thong district health promoting hospital. Gathered information concerning of pesticide exposures from 50 rice farmers and 50 control respondents was conducted by questionnaire interviewing. Each serum sample was determined SChE activity test by paper test and automatic analyzer; and EDTA blood was analyzed genotyping by PCR-RFLP. Chi-square was used to analyze on the different of personal information; and of polymorphism. Person correlation was evaluated relationship between SChE level and genotypes. 60% of rice farmers were used pesticide over 10 years. Means of SChE level of both groups were within reference value and significantly different ($p = 0.033$). Polymorphisms were included wild type (GG), heterozygote (GA) and homozygote (AA); and were significantly different between rice farmers and control ($p = 0.0001$). GA genotype in rice farmers was more frequent and AA genotype was not detected. Relationship of polymorphism and SChE level were negative correlated ($r = -0.258$; $p = 0.009$). CYP2D6 gene polymorphism may useful biomarker for duties assignments in agricultural workers especially risk group (GA genotype).

***Keywords**: CYP2D6 gene polymorphism, cholinesterase inhibitors, chronic pesticide exposure, cytochrome P450, organophosphate, serum cholinesterase*

Introduction

Pesticides are substances that are meant to control pests, including weeds. The most common type of pesticides imported in Thailand is herbicides, followed by insecticides and fungicides [1] (Ministry of Agricultural and Cooperatives Office of Agricultural Economics, 2014). Thai agricultural workers report they typically use a variety of pesticides, applying them an average of three to four times a month [2, 3]. Suphanburi province is located in central plain, which is a member of “rice bowl” of Thailand. Because of enough water supplies in this cultivation area, two or three rice growing cycles can be done rather than Northeast area. Rice growing cycle is consisting of land preparation, seeding, planting and harvesting. Agrochemicals for

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seeding and planting period are more use in Central area than in Northeast area, thus, farmers may expose to pesticides more frequent rather than other area also. Moreover, agropesticide application in Central of Thailand is on intensively cultivated rice crop land when water conditions allow had been occurred [4]. This is a significant factor in allowing many farmers to continue growing rice, but it has also created health hazards for those who took up agropesticide spraying as a profession [5]. Agricultural workers in Suphanburi had reported using several brands of insecticides, plant hormones, and chemicals for "control of plant diseases". Most of active insecticide substances in commercial products are abamectin, chloropyrifos, carbofuran, and cypermethrin [6, 7].

Chloropyrifos and carbofuran are common members of organophosphate (OP) and carbamates (CB), respectively. OP and CB act as acetyl cholinesterase (AChE) inhibitors, which are affecting 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 [8-10]. Biological effects of pesticide are involved by oxidative stress, epigenetic controls and gut microbiological digestion and modulation of genetic polymorphisms [11]. Organophosphate pesticides (OPs) are primarily metabolized by several xenobiotic metabolizing enzymes, which are including cytochrome P450 enzymes. Cytochrome P450 polymorphisms are common biomarkers for evaluation of susceptibility in pesticide exposure [12]. CYP2D6 G1934A (rs 3892097) gene polymorphism is leads to catalytic rate of enzyme activity [13]. CYP2D6 1934A allele is related to the susceptibility to organophosphate chronic toxicity in Egyptians [14]. This study was aimed to investigate CYP2D6 G1934A gene polymorphism in Thai rice farmers along with occupational health risks and behaviors; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. This genotypic polymorphism may help to identify the risk of occupational exposure especially management of chronic pesticide toxicity.

Materials and Methods

Subject recruitment and data collection

The study was conducted in U-Thong district, Suphan Buri province, about 150 km from West of Bangkok, where rice is main harvesting product. Main of herbicide use is paraquat and glyphosate. Outsource pesticide sprayers were common finding in rice field. 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, such as, liver diseases, severe cardiovascular diseases, cancer 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, personal protective equipment (PPE) used, and practical knowledge 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 [3]. The Ethics Committee of Thammasat University was approved this research protocol (COA No. 085/2562). 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 [15] 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 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 [16]. 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.

CYP2D6 G1934A polymorphisms by PCR-RFLP

DNA template was amplified by polymerase chain reaction (PCR) using forward 5'-GCT TCG CCA ACC ACT CCG-3' and reverse 5'-AAA TCC TGC TCT TCC GAG GC-3' primers, which were corresponded to CYP2D6 G1934A region [17]. PCR was performed in 25 µl of total volume, which was contained 1 µl of DNA template, 0.5 µl of each primer (20 pmol/µL), 5 µl of 10X PCR buffer (1.5 mM Mg^{2+}) include dNTP mixture and 0.2 µl of 1 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 1 min, annealing at 61°C for 1 min and extension at 72 °C for 1 min followed by final extension at 72 °C for 7 min by using Thermal cycler (Applied Biosystems, USA). Restriction fragment length polymorphism (RFLP) was performed: DNA product was digested by *BstNI* 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). CYP2D6 G1934A single nucleotide polymorphisms (SNPs) were 1) 104- and 230-bp fragments for the 1934G allele and 2) 334-bp fragment (undigested) for the 1934A allele. The interpretation of CYP2D6 G1934A genotypes were represented 104, 230-bp fragment for wild type (GG); 104, 230, 334-bp fragments for heterozygous (GA); and 334-bp fragments for homozygous (AA) [14]. Quality control of test was done by DNA sequencing, which was randomized from 15% of samples.

Statistical Analysis

Descriptive data was explained by using mean and standard deviation; and frequency. The Kolmogorov-Smirnov test was used to test for normal distribution of data. Chi-square was used to analyze on the different of personal information and CYP2D6 G1934A genotypes between rice farmer and controls groups. Independent *t*-test was used for comparison of SChE level between rice farmer and controls groups. Person correlation was evaluated between SChE level and PON1 Q192R 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

Chronic pesticide exposure in rice farmers

No statistical significance different between rice farmers and controls for gender and risked behavior, such as alcoholic consumption. 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 and almost of them had health education for awareness of pesticide uses (Table 1). However, 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).

Some of rice farmers had SChE level lower than reference value (data not show), which was implied that SChE 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 [18]. Most of studies on Thai pesticide exposure had reported exposure of single type of pesticide; however, mixed pesticide uses are more common for multi-crop cultivation. Thus, screening of other biomarkers for evaluation of long-term pesticide exposure rather than serum cholinesterase such as, alkyl phosphate metabolites (DAPs), urinary 3-phenoxybenzoic acid (3-PBA) and urinary glyphosate, is still necessary for public provider [19].

CYP2D6 (G1934A) gene polymorphism in rice farmers

The DNA fragments electrophoresis of CYP2D6 (G1934A) gene polymorphism in rice farmers were included wild type (GG), heterozygote (GA) and homozygote (AA) (Fig. 1). The genotypic polymorphisms of rice farmers and control were significantly different (p=0.0001); rice farmers were more frequent RR genotype rather than control (Table 2), however AA genotype was not detected from both groups. The relationship of CYP2D6 (G1934A) gene and SChE level were negative correlated (r = -0.258) with statistically significant (p = 0.009) (Table 3). Six of rice farmers were low SChE level (<5,500 U/L) and GA genotype polymorphism (data not show).

CYP2D6 is important member of cytochrome P450 based on various xenobiotic substrates, which are shows a very high degree of variability due to the extensive genetic polymorphism that influences gene expression and enzymatic function [20]. CYP2D6 gene is involved in the secondary metabolic pathway of desulphuration of isocarbophos (ICP). ICP enantiomers and its oxidative desulphuration metabolite, isocarbophos oxon (ICPO) has been reported to be inhibitors of acetylcholinesterase [21, 22]. The CYP2D6 inhibitors are resulted in 50% inhibition of cholinesterase activity for parathion, 38% diazinon and 30% chlorpyrifos as compared to control [23] and SChE is significantly reduced in chronic organophosphate exposures [14]. The variants of CYP2D6 alleles at third intron and four exons are resulted in missing of transcription and single based deletion of mRNA [24]. Thus, this finding was corresponded to previous study by reduction of cholinesterase activity and GA genotype was more susceptible to pesticide rather than wild type in Thai population, which was sparely reported. Large scale and seasonal variation studies on gene polymorphism and environmental expose will conduct to confirming the finding. Genotyping on polymorphism may also useful biomarker for agricultural workers to assignment of appropriate duties, especially high risk group.

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)	-	

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

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: Genotypic frequency of CYP2D6 (G1934A) in rice farmer and control groups

Genotype	Control		Farmer		p-value
	N	%	N	%	
Wild Type (GG genotype)	44	88	22	44	0.0001*
Polymorphism (GA genotype) a	6	12	28	56	

* Statistically significant at $p<0.05$

[a] Homozygous polymorphism (AA genotype) was not detected

Table 3: Relationship of CYP2D6 (G1934A) genotypes and SChE level

Genotype	N	SChE (U/L)	r	p-value
Wild Type	66	7820.14 ± 1103.25	-0.258	0.009*
GA Polymorphism	34	7278.89 ± 1307.70		

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

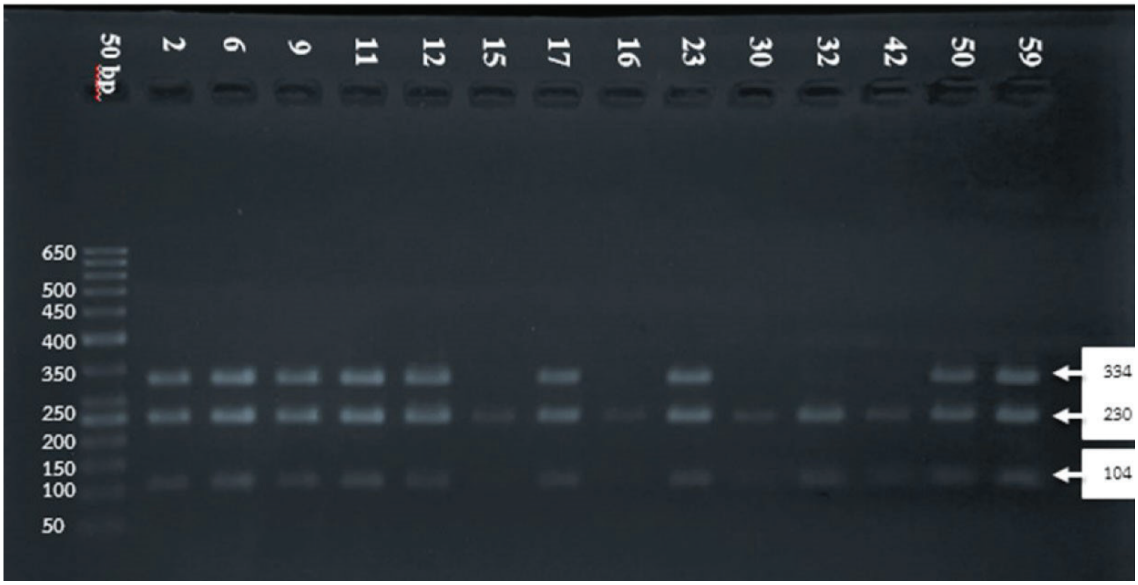

Figure 1: DNA fragment separation. First Lane (from left): DNA ladder; Lane 2, 6, 9, 11, 12, 17, 23, 50 and 59: 104, 230-bp for GA genotype (polymorphism); and Lane 15, 16, 30, 32 and 42: 334-bp for GG genotype (wild type).

Conclusion

CYP2D6 (G1934A) polymorphism may useful biomarker combining with SChE activity test for duties assignments in agricultural workers especially risk group (QR genotype).

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, Bangkok, Thailand.

Ethical Clearance: Ethics Committee of Thammasat University was approved this research protocol (COA No. 085/2562).

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

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