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PON1 L55M GENE POLYMORPHISM AND SERUM PON1 LEVEL IN THAI RICE FARMERS EXPOSED ORGANOPHOSPHATE

Wanida Pongstaporn[1*], Kanyanan Kritsiriwuthinan [2] and Yuttana Sudjaroen[3]

[1]Pathobiology unit, Department of Medical Sciences, Faculty of Science, Rangsit Unviersity. Pathumthani 12000,Thailand
[2]Faculty of Medical Technology, Rangsit University, Pathumthani 12000 Thailand
[3]Department of Applied Sciences, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Thailand

*Corresponding author : E-mail : wanida.po@rsu.ac.th
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Abstract: Paraoxinase 1 (PON1) involves in the detoxification of organophosphate compounds . Polymorphisms of the PON1 gene are responsible for variation in the expression and catalytic activity of PON1 .This paper aims to investigate PON1 L55M genotypes in Thai rice farmer who exposed organophosphate. Moreover, PON1 enzyme level was compared between Thai rice farmer whose exposed organophosphate and control subjects. This study revealed that the wild type of PON1 L55M is the majority of agricultural worker who exposed organophosphate (48 out of 50 cases) and serum PON1 level is statistically higher in agricultural worker who exposed organophosphate than in control subjects (p=0.04).

Keywords: Paraoxinase1, organophosphate, PON1 L55M, PON1 enzyme level

INTRODUCTION

Thailand is the country where agricultural is main occupation. Over the last decade, the pesticides are used to increase productivity, especially in rice farmer. About 3% of agricultural workers in developing Asian countries suffer from pesticide poisoning every year. Organophosphate (OP) is the most common pesticides used in agricultural. Organophosphate toxicity may occur due to exposure directly or through dispersion of substance (O'Malley, 1997).

Paraoxonases are a family of three enzymes called PON1, PON2 and PON3 .They have multifunctional roles in several biochemical pathways such as protection against oxidative damage and lipid peroxidation, innate immunity, detoxification, bioactivation of drugs, and regulation of cell proliferation and apoptosis (Martinelli et al., 2013).

PON1 is the most studied enzyme of the family .It is synthesized primarily in the liver and appears mainly in serum, where is associated to high-density lipoproteins (HDL). PON1 involves in the detoxification of organophosphate ester by hydrolyzing bioactive oxons (Costa et al., 2003). There are more than 200 single nucleotide polymorphism of PON1 (Jarvik et al., 2000) .Some SNPs of PON1 may be related to affect the quality and quantity of PON1 .

There are two SNPs of PON1 that may be related to PON1 activity and concentration including PON1 Q192R and PON1 L55M.

Polymorphisms of the PON1 gene are responsible for variation in the expression and catalytic activity of PON1. Serum PON1 level and activity varies among different ethnic population. There are several evidences have demonstrated that functional polymorphisms in

the PON1 gene might play a critical role in increasing susceptibility to organophosphate toxicity, but individually published studies showed inconclusive results. PON1 Q192R and PON1 L55M are two common PON1 gene polymorphism (Shunmoogamm et al., 2018). The role of PON1 Q192R polymorphism is involved in catalytic efficiency of PON1, while PON1L55M affects PON1 mRNA and serum PON1 concentration (Humbert et al., 1993).

From the previous study, PON1 Q192R polymorphism was investigated in rice farmers exposed organophosphate form U-thong district, Thailand. It was found that Thai rice farmers were significantly higher frequency of PON1 RR genotype than in the control group ($p= 0.02$). Serum pseudocholinesterase (SChE) in Thai rice farmers was significantly lower than the control group ($p= 0.033$). Relationship of polymorphism and SChE level were negative correlated ($r = -0.261$; $p = 0.031$) (Sridon et al.,2020).

PON1 L55M polymorphism is another polymorphism that may be involved in susceptibility of organophosphate toxicity. There is no research about PON1 L55M polymorphism in Thai rice farmers whose exposed organophosphate.

This paper aimed to investigate PON1 L55M genotypes in Thai rice farmer who exposed organophosphate. Moreover, PON1 enzyme level was compared between Thai rice farmer whose exposed organophosphate and control subjects.

MATERIALS AND METHODS

Specimen collection

This is the cross sectional study .The samples comprised of DNA and serum from sample bank of the previous study (Sridon et al., 2020) .This study comprised of 100 respondents included two groups .The first group composed of 50 rice farmers (risk group) who lived in U-thong district area and had handle pesticide regularly at least three years or more and the second group is 50 control subjects who lived nearby field area and had non related professional for farm workers .DNA was extracted from EDTA blood and serum was obtained from clotted blood of 50 Thai rice farmers whose exposed organophosphate and 50 Thai control subjects .

The study was approved by the Ethics committee of Thammasat University (COA No . 084/2562) and the Ethics committee of Rangsit University. (COA :RSUERB2020-005)

The director of U-thong district health promoting hospital, Suphan Buri province, gave permission to do the research . Inform consent was obtained from all the participants.

Because this paper aimed to investigate PON1 L55M genotypes in Thai rice farmers of U-thong district whose exposed organophosphate and compared the PON1 enzyme level in rice farmers of U-thong district compared with control subjects .Therefore, PON1 L55 M polymorphism was investigated in 50 cases of Thai rice farmer in U-thong district .Serum PON1 level was compared between 46 cases of Thai rice farmers and 28 cases of normal control subjects.

DNA extraction

Genomic DNA in 50 Thai rice farmers and was isolated from EDTA blood by QIAamp blood DNA mini kit (QIAGEN, New England Bio-Labs, Waltham, MA, USA) . The DNA purity and concentration was determined by spectrophotometer measurement of absorbance at 260 and 280 nm .Genomic DNA was stored at -20^0C .Using genomic DNA, PON1 L55M polymorphism was identified by polymerase chain restriction fragment length polymorphism (PCR-RFLP).

PON1 L55M genotyping by PCR-RFLP

Genotyping of PON1 L55M polymorphism was investigated by PCR-RFLP as described previously (Singh et al., 2011).

The 172 bp DNA fragment containing the polymorphic site was amplified using 5'CCT GCA ATA ATA TGA AAC AAC CTG-3', and 5 'TGA AAG-ACT-TAA-ACT-GCC-AGT-C-3' as forward and reverse primer, respectively .The PCR cycling conditions were the initial denaturation of 94^0 C for 5 min followed by 35 cycles of 94^0 C for 1 min, annealing at 61^0C for 1 min, and 72^0 C for 1 min, with a final elongation at 72^0 C for 7 min .The PCR products were identified by electrophoresis on 2 %agarose. The PCR product sizes, after restriction digested with *Nla*III (New England Bio-Labs, Waltham, MA, USA) at 37^0C overnight .Genotyping was assessed by electrophoresis on 2.5% agarose gel .The restriction fragments include a single 172 bp fragment for the LL genotype (homozygous wild type), two fragments of 106 and 66 bp for the MM genotype (homozygous variant) and three fragments of 172, 106 and 66 bp for the LM genotype (heterozygous).

Quality control of test was done by DNA sequencing in 30 cases of Thai rice farmers whose exposed organophosphate.

Determination of serum PON1 levels by ELISA

Serum from clotted blood of 46 Thai rice farmers and 28 Thai control subjects was used for the determination of serum PON1 levels by ELISA method (QuickDetect^{TM} PON1 Human ELISA Kit, BioVision, USA).BioVision's PON1 ELISA kit is a sandwich ELISA assay for quantitative measurement of human PON1 in serum. The density of color is proportional to the amount of human PON1 captured from the samples. The comparison of serum PON1 level between 46 cases of Thai rice farmers and 28 cases of normal control subjects was assessed by Independent sample T-test.

RESULTS AND DISCUSSION

PON1 L55M genotyping in Thai rice farmers

The PCR products of PON1 L55M are 172 base pairs .PON1 L55M genotypes were identified by PCR-RFLP method .The result of genotyping was confirmed by DNA sequencing method. It was found that in 50 cases of Thai rice farmers whose exposed organophosphate, there are 48 cases of wild type and 2 cases of homozygous polymorphism.

The genotypes of PON1 L55 M detected by PCR-RFLP were shown in figure 1.

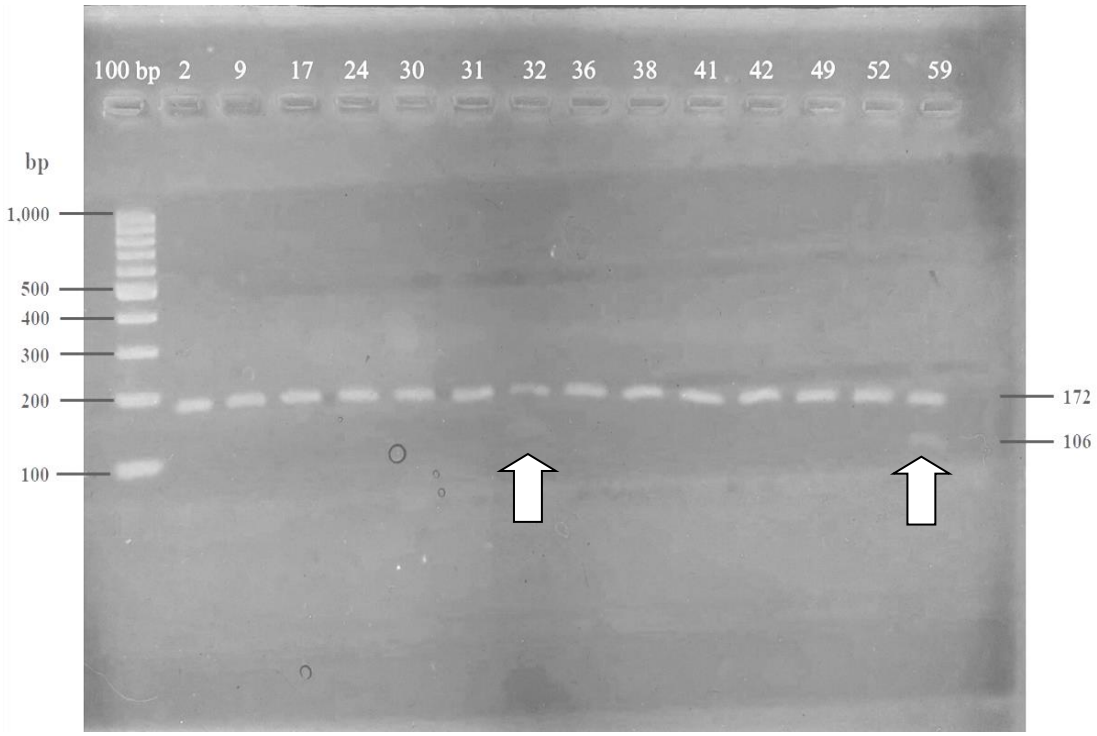

Figure 1. The example of PCR-RFLP pattern in genotyping of PON1 L55M. Genotyping in almost every number of subjects were wild type genotypes, except number 32 and number 59 which showed band with 172 bp and 106 bp as shown by pointer (white pale band).

The database of DNA sequence in PON1 L55M (rs854560) was shown in figure 2.

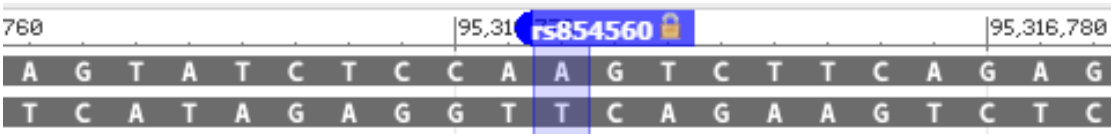

Figure 2. The database of DNA sequence in PON1 L55M (rs854560).

The result of DNA sequencing in number 38 was shown in figure 3.

The result of DNA sequencing of number 32 and number 59 were the same pattern of the homozygote polymorphism (PON1 LL) as shown in figure 4.

Figure 3 The result of DNA sequencing in number 38 was wild type (PON1 LL).

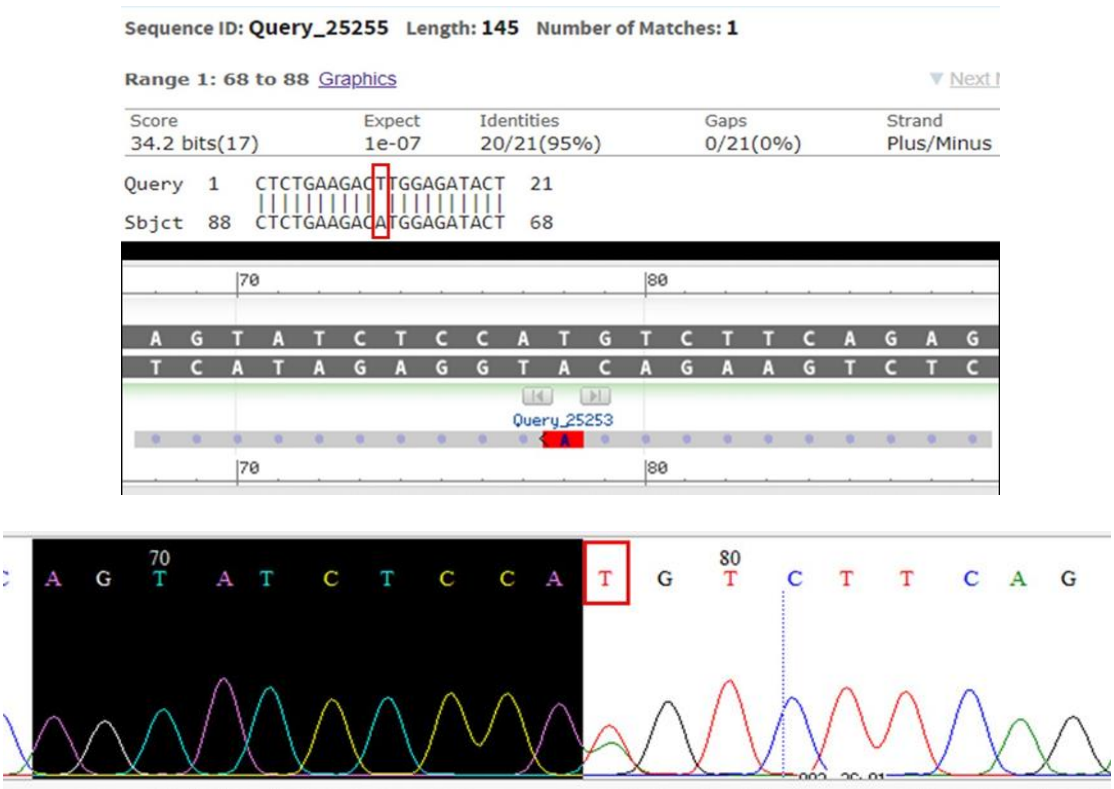

Figure 4 The result of DNA sequencing in number 32 and 59 were homozygote polymorphism (PON1 MM).

The results of sequencing from 30 cases of Thai rice farmers in U-thong district whose exposed organophosphate were 28 cases of wild type genotypes and two cases of homozygous variants.

Serum PON1 levels by ELISA

The result of serum PON1 level between 46 cases of Thai rice farmers compared with 28 cases of normal control subjects as shown in Table 1.

Table 1 Serum PON1 level in 46 cases of Thai rice farmer compared with 28 cases of normal control subjects.

Group	N	Enzyme PON1 level (Mean± SD) (U/L)	*P-value*
Control	28	7.52 ±1. 58	**0.04***
Farmer	46	826. ±1. 68	

* Independent sample T-test.

Serum PON1 level in Thai rice farmer is statically significant higher than control subjects (p=0.04).

Recently, it was found that polymorphisms of PON1 Q192R (QR+RR) was statically higher in Thai rice farmer than control subjects (p=0.02) (Sridon, et al.,2020).

From meta-analysis study, it was found that the PON1 192Q and 55L polymorphisms may increase the risk of organophosphate toxicity .Further subgroup analyses by ethnicity showed significant associations of the PON1 192Q and 55L polymorphisms with increased risk of organophosphate toxicity among the Caucasian populations .However, similar association was not observed among the Asian populations (You et al., 2013).

PON1 L55M is another gene polymorphism of PON1 that related to PON1 level in serum. Until now, there are no report of PON1 L55M gene polymorphism in Thai rice famer exposed organophosphate .In this study, the results of PON1 L55M genotyping in 50 cases of Thai rice farmer found that there are only two cases whose genotyping is homozygous polymorphism of PON1 L55M (MM genotype). The result of PCR-RFLP was confirmed by DNA sequencing in 30 out of 50 cases of Thai Rice farmer .However, this study is in accordance from the meta-analysis study of You et al. (2013) which found that PON1 L55M was not observed in Asian population .It was found that about 80 %of Thai rice farmers in U-thong district, Suphanburi had already known the effects of pesticide to health status and 66 % of Thai rice farmers had personal protective equipment .From the previous study (Sridon et al, 2020), there are only 6 cases of Thai rice farmers who have low level of acetycholinesterase. The low level of acetylcholinesterase is the risk factor for organophosphate toxicity .However, PON1 L genotype which is wild type was found in all of these 6 cases .Therefore, PON1 L55M is not involved in organophosphate toxicity in Thailand .

In the previous study, it was found that PON1 L55M correlates to PON1 in serum (Brophy et al, 2001). Serum PON1 level is determined by ELISA method .It is already known that the study of PON1 activity is the best investigation of PON1 .However, due to the serum is left over for the long time .Therefore, the study of activity cannot be performed because the problem of enzyme stability .The serum concentration of PON1 is another alternative way to investigate PON1 level in this study .

The results showed that the serum PON1 level in Thai rice farmer exposed organophosphate is statistically higher than control group (p=0.04). However, most of all cases enzyme PON1 level is in the normal range .This normal value of enzyme PON1 was correlated to the genotype of PON1 L55M which showed wild type in almost cases .This is

the reason that why Thai rice farmer who exposed organophosphate is still asymptomatic in most cases.

CONCLUSION

In conclusion, this study found that PON1 L55 M genotype is the wild type in majority of agricultural worker who exposed organophosphate and serum PON1 level is statistically higher in agricultural worker who exposed organophosphate than in control subjects (p=0.04) but serum PON1 level is in the normal range. This normal value of enzyme PON1 was correlated to the wild type genotype of PON1 (LL) in almost cases. This is the reason that why Thai rice farmer who exposed organophosphate is still asymptomatic in most cases.

ETHICS STATEMENT

This study was approved by the Ethical Clearance Committee on Human Right Related to Research Involving Human Subjects Ethics committee of Thammasat University (COA No. 084/2562) and the Ethics committee of Rangsit University. (COA : RSUERB2020-005).

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REFERENCES

Brophy VH, Hastings MD, Clendenning JB, Richter RJ, Jarvik GP, Furlong CE. 2001. .Polymorphisms in the human paraoxonase (PON1) promoter .*Pharmacogenetics* .11(1):77-84 .doi: 10.1097/00008571-200102000-00009.

Costa LG, Giordano G, Cole T, Marsillach J and Furlong CE . 2013 .Paraoxinae 1(PON1)as a genetic determinant of susceptibility to organophosphate toxicity .*Toxicology*. 10; 115-122. doi:10.1016/j.tox.2012.07.011.

Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE .1993. The molecular basis of the human serum paraoxonase activity polymorphism .*Nat Genet*.76–73:(1)3 . DOI: 10.1038/ng0193-73.

Jarvik GP., Rozek LS., Brophy VH., Hatsukami TS., Richter R.J., Schellenberg GD ., Furlong CE. .2000 Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is *PON1* 192 or *PON1* 55 genotype .*Arterioscler* .*Thromb* .*Vasc* .*Biol* .20, 2441–2447. doi: 10.1161/01.atv.20.11.2441.

Martinelli N, Consoli L, Girelli D, Grison E, Corrocher R, Olivieri O. 2013. Paraoxonases :ancient substrate hunters and their evolving role in ischemic heart disease .*Adv Clin Chem*, 59: 65–100. DOI: 10.1016/b978-0-12-405211-6.00003-6

O'Malley M. 1997 .Clinical evaluation of pesticide exposure and poisonings .*Lancet* 349)9059:(1161–1166, DOI:https://doi.org/10.1016/S0140-6736 (96)07222-4.

Shunmoogam N, Naidoo P., Chilton R.. 2018. Paraoxonase (PON-1) :a brief overview on genetics, structure, polymorphisms and clinical relevance. *Vasc Health Risk Manag* 14 :137–143 .doi : 10.2147/VHRM.S165173.

Singh S et al2011 .. Roles of genetic polymorphisms of CYP1A1, CYP3A5, CYP2C9, CYP2D 6and PON 1in the modulation of DNA damage in workers occupationally exposed to organophosphate pesticides . *Toxicology and Applied Pharmacology* 257; .92-84 doi: 10.1016/j.taap.2011.08.021.

Sridon A., Ruchiwit K., Suwannahong K., Pongstaporn W., Sudjaroen Y .2020 .Pon1 q192r gene polymorphism and pesticide exposure status of rice farmers, Suphan Buri, Thailand .*Indian J of Forensic Medicine and Toxicology*. 12; (2) :152-157.

Tian You, Jingyu LV and Liping Zu .2013 .PON1 Q192R and L55M polymorphisms and organophosphate risks : A meta-analysis .*DNA and Cell Biology* 32; (5) :252-259. DOI: 10.1089/dna.2012.1961.