

# GENETIC VARIANTS OF HUMAN BUTYRYLCHOLINESTERASE AND ITS POTENTIAL IMPACT ON EGYPTIAN FARM WORKERS ACUTELY EXPOSED TO ORGANOPHOSPHATE PESTICIDE

By

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## Abstract

**Introduction:** Organophosphate compounds (OPs) are potent and effective insecticides widely used in human practice. They are irreversible cholinesterase inhibitors causing accumulation of acetylcholine at cholinergic synapses with over-stimulation of muscarinic and nicotinic receptors. In Egypt, little is known about susceptibility to toxic effects of OPs during pesticide application. **Aim of work:** To study the genetic variation in Butyrylcholine esterase (BChE) among workers presented to the National Egyptian Centre of Toxicological and Environmental Research (NECTR) with acute toxicity during spraying of organophosphorus pesticide and having occupational history of chronic exposure. **Materials and methods:** It is a case-control study to assess BChE genotype and enzyme activity in 65 workers presented to NECTR at Cairo, Egypt, during the period from January 2014 to July 2016. Comparison with 55 matched control subjects was performed; to identify those workers at risk to potential adverse health effects of OP compounds. The studied group answered a predesigned questionnaire with detailed personal medical and occupational histories. Full physical examination was done. Laboratory investigations included: liver and kidney functions, analysis of pseudo choline esterase and DNA. **Results:** The study revealed that 73.8 % of the total cases was carrying JK variants (named in honor of James and Kalow respectively) in heterozygote state with the A (Atypical) variant and 13.8 %

in heterozygote state with the U (Usual) variant, these workers showed reduction in the level of BChE enzyme. **Conclusion:** BChE genetic variations could be a concern in farm workers exposed to organophosphorus pesticides in agriculture and could be useful in assessing the risk of pesticide exposure. Safety regulations concerning products use, training of occupational workers for the safe application of potentially harmful pesticides are highly recommended.

**Key words:** Organophosphorus compounds, Butyrylcholine esterase, A (Atypical) variant, U (Usual) variant, Genetic susceptibility, Farm workers and Safety regulations.

## Introduction

Organophosphate compounds (OPs) are potent and effective insecticides widely used and still represent the largest group of insecticides sold worldwide. These compounds are responsible for the million of poisonings and thousands of deaths occurring annually particularly in third world countries (Costa, 2006).

Organophosphate compounds (OPs) are potent irreversible cholinesterase (ChE) inhibitors causing accumulation of acetylcholine at cholinergic synapses with over-stimulation of muscarinic and nicotinic receptors (Grupta and Bechan, 2016). There are two types of cholinesterases in the human body: acetylcholinesterase and butyrylcholinesterase [BChE, plasma cholinesterase, or pseudo-cholinesterase]. BChE acts as scavenger of the highly toxic OPs and nerve agents (Tabun, Sarin, Soman) (Raveh et al., 1997). Its measurement is useful as a primary biomarker in emergency medicine in cases of poisoning and ac-

cidental organophosphate or carbamate exposure (Simoniello et al., 2010).

Occupational pesticide (Ops) handlers are at high risk for intoxication. Mixing or applying these pesticides can result in potentially harmful levels of exposure through one or more high exposure events or through chronic lower-level exposure (Strelitz et al., 2014). The degree of generated damage depends on the individual health status and sensitivity to the particular pesticide (Gaikwad et al., 2015).

Because of this risk, an important question raised on, why some personnel developed acute symptoms after exposure while others with similar exposures remained healthy or asymptomatic. One possibility is that vulnerability to certain work-related exposures differed as a result of individual variability in the biological processes that neutralize these exposures and confer protection from acute and/or chronic adverse effects (Steele et al., 2015).

So, investigators have begun to examine the link between exposures to organophosphorus pesticides and genetic variation among farm workers and other agricultural workers (Furlong et al., 2005; Furlong et al., 2006; Holland et al., 2006).

More than 100 polymorphisms of BChE had been identified (Shields and Lewis, 2011). Besides the Usual (U) variant of BChE ; several genetic variants with altered enzyme activity have been reported, such as the Atypical gene (A), Fluoride resistant gene (F), Silent gene (S), K variant (Kalow), J variant (Jame) and H variant (Hammersmith) (Masato et al., 1997) . Many surveys indicated that an individual's genotype carrying some of these variants may result in higher sensitivity to organophosphorus compounds (Howard et al., 2010).

However, a comprehensive analysis of the genetic variation, combined with a thorough collection of environmental and behavioral data in farm workers exposed to pesticides has not been performed to date.

Occupational health risk evaluation is a priority because alterations in the genetic material could result, leading to possible development of diverse pathol-

ogies, including cancers and neoplastic diseases (Yaduvanshi et al., 2012).

### **Aim of work**

To study the genetic variation in Butyrylcholine esterase in workers presented to the National Poison Centre of Toxicological and Environmental Research (NECTR), Cairo University, with acute toxicity during spraying of organophosphorus pesticide and having occupational history of chronic exposure.

### **Material and methods**

**Study design:** It is a case-control study

**Place and duration of the study:** The study was conducted in the National Poison Centre of Toxicological and Environmental Research (NECTR), Cairo University, Cairo, Egypt, during the period from January 2014 to July 2016.

**Study sample:** The target group was workers presented to NECTR with acute exposure to organophosphorus compounds during spraying. They were 65 male workers with age ranging from 18 to 59 years with a mean value of  $26.92 \pm 9.69$ , they were compared with 55 employees working in administrative offices in NECTR and in the Bio-

chemistry department, they were male subjects not exposed to any pesticides with age ranging from 19 to 55 years with a mean value of  $27.22 \pm 9.31$ . Both groups were matched regarding age, sex, socioeconomic status and special habits with no statistical significant difference between them.

**Study method:** All workers were presented to the emergency room in NECTR and properly managed. After full stabilization of the condition:

- They were personally answered a predesigned questionnaire with detailed personal medical and occupational histories including methods and duration of exposure to OPs. Pesticide sprayers participating in this study did their work with scarce use of safety protective measurements. The workers reported exposure to OP during the spraying season for 2 to 3 hours daily.

- **Full physical examination** was done.

- **Laboratory investigations:**

### 1- Blood sample collection:

Ten ml of venous blood were taken from the studied groups under aseptic conditions:

- Three ml was put into a tube containing Ethylene Diamine Tetraacetate (EDTA) for genetic analysis.
- Two ml of blood were centrifuged for serum separation and plasma pseudocholine esterase analysis.
- Five ml of blood were left to clot, centrifuged for serum preparation and chemical analysis which was performed using the photometer PM 750 for measurement of SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase) (normal values up to 12 U/L), serum creatinine (normal value = 0.5 -1.2 mg%) and blood urea (normal value = 19-50 mg%).

### 2- Pseudo choline esterase analysis

Three reagents were used (R1 = buffer pyrophosphate, R2=DTNB , R3=BTC). We add one cm of R2+Buffer to 100 UL of R3+Buffer to 5 UL of serum , they are mixed carefully and measured after 30 seconds using the spectrophotometer at wave length 405 nm. (Normal value  $\geq 3000$ - 1900 UL).

### 3- DNA Analysis:

**A- DNA Amplification and Sequencing:**

White-blood-cell DNA from 120 subjects (65 exposed and 55 control) was purified from whole blood and amplified by Polymerised Chain Reaction (PCR) using primers directed toward introns, noncoding flanking regions of the gene, or within the coding region. End-labeled primer was combined with amplified DNA in four reaction mixtures: denaturation, annealing, incubation and extension of the annealed primers with the DNA polymerase (Bartels et al., 1992). Then selected areas of the BChE gene were sequenced. DNA representing the entire coding sequence of the enzyme, contained in exons 2, 3, and 4, was amplified and PCR products were analyzed from agarose gel electrophoresis.

#### **B-Digestion of Amplified DNA with RsaI:**

The J-variant mutation created an RFLP in exon 3. Exon 3 DNA from individuals of various BChE phenotypes was amplified and digested with RsaI. Approximately 400 ng of amplified DNA (one-fifth of the total amplification reaction) was digested in buffer L (10 mM Tris-HCl pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM dithioerythritol [DTE]; Boehringer-Mannheim) supplemented with 1.2 mM DTT and 40 units of RsaI

in a total of 85  $\mu$ l. Electrophoresis was performed on a 25-30  $\mu$ l sample of the digest loaded onto a 2% agarose gel.

#### **C- RFLP Analysis of the J-Variant Point Mutation:**

The J-variant DNA mutation (Glu497--Val), created an RsaI RFLP. DNA of individuals exhibiting the J-variant phenotype was amplified in the exon 3 regions by using amplification primers directed toward introns 2 and 3. The amplified DNA fragment was 336 bp, resulting from exon 3 (167 bp), 87 bp of intron 2, and 82 bp of intron 3. RsaI digests of amplified DNA from individuals heterozygous for the J-variant showed three bands: the uncut band of 336 bp from the non-J variant allele, as well as two bands, one of 192 bp and the other of 144 bp, from the J-variant allele. Similarly amplified and digested DNA from individuals whose serum phenotype did not indicate the J-variant did not show this RFLP. No individuals homozygous for the J-variant were available.

#### **Consent**

A written consent was taken from the patients or their relatives- according to the age and the level of consciousness- to be involved in the research.

They were informed that all collected data will be confidential and used for scientific purposes only.

### Ethical approval

The study was approved by director of NECTR, and the administrative authority of Kasr Al Ainy Hospital, Faculty of Medicine, Cairo University.

### Data management

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data was summarized using mean, standard deviation, median, mini-

mum and maximum in quantitative data and using frequency and percentage for categorical data. Comparison between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003 a). For comparing categorical data, Chi square ( $\chi^2$ ) test was performed. Exact test was used instead when the expected frequency is less than 5 (Chan, 2003 b). Correlations between quantitative variables were done using Spearman correlation coefficient (Chan, 2003 c). p-values less than 0.05 were considered as statistically significant.

## Results

**Table (1): Comparison between exposed and non exposed regarding genetic analysis**

Studied groups		Exposed		Non exposed		Test of significance	p- value
		No	%	No	%		
Genetic analysis	A/AK	0	0%	38	69.10%	$X^2=98.709$	
	A/JK	34	52.30%	0	0%		< 0.001**
	AK/JK	14	21.50%	0	0%		
	U/A	6	9.20%	15	27.30%		
	U/JK	9	13.80%	0	0%		
	U/U	2	3.10%	2	3.60%		

\*\* Highly statistically significant

The 120 subjects involved in this research were arranged into six groups according to their BChE genotypic variants.

Table (1) showed that most of the exposed workers had the Atypical genotype (A) heterozygotes with J and K variants [A/JK → 52.3%; AK/JK → 21.5% (total=73.8%)], the percentage of genotypes corresponding to U-heterozygotes; with the A variant (9.2 %) and with the JK variant (13.8 %). The exposed workers carrying JK variants with A and U = 87.6%. Only two individuals (3.1 %) from the exposed group were detected with the normal genotype corresponding to UU.

Table (1) also showed that the majority of the non exposed group (69.1%) had the abnormal genotypes (A/AK). The percentage of genotypes corresponding to U-heterozygotes was 27.3 % of individuals. Only two individuals (3.6 %) from the non exposed group were detected with the usual genotype corresponding to UU.

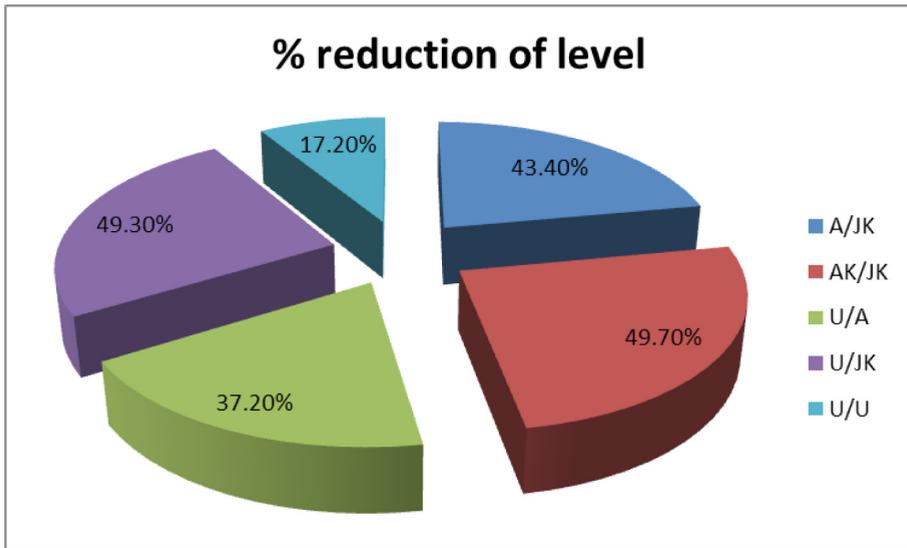
**Table (2): Relation between cholinesterase levels and different genotypes among exposed group.**

Exposed A/JK (No=34)		Genetic analysis					Test	p- value
		AK/JK	U/A	U/JK	U/U	Non parametric		
		(No=14)	(No= 6)	(No=9)	(No= 2)	Kruskal- Wallis		
Chol Es	Mean	1700	1539.93	1884.17	1523.11	2185.21	8.144	0.086
	Standard Deviation	282.84	293.19	544.43	401.61	1284.38		
	Minimum	1500	1000	1100	800	900		
	Maximum	1900	2000	2700	2248	6700		

Chol Es: Choline-esterase

Table (2) showed the relation between cholinesterase levels and different genotypes among exposed group which revealed that the lowest levels were recorded in those carrying the J variant genotype.

**Graph (1): Percentage reduction of cholinesterase level in relation to different genotypes among the exposed group.**



Graph 1 showed the percent reduction of cholinesterase level (calculated from 3000 level - which is the cutoff point of cholinesterase level) in relation to different genotypes among workers.

**Table (3): Relation between cholinesterase levels and different genotypes in non exposed group.**

Non exposed group A/AK (No = 38)		Genetic analysis			Test	p- value
		U/A (No = 15)	U/U (No = 2)	Non parametric Kruskal- Wallis		
Chol Es	Mean	3186.84	3010	5450		<b>0.049*</b>
	Standard Deviation	737.75	619.97	212.13	6.047	
	Minimum	1500	1900	5300		
	Maximum	4600	4200	5600		

Chol Es: Choline-esterase

\* Statistically significant

Table (3) showed that the highest levels of BchE in the control group were recorded in those with the usual variant UU with significant difference between the different genotypes.

**Table (4): Comparison between exposed and non exposed groups regarding laboratory investigations.**

	Exposed		Non exposed		Z test	p- value
	Mean	SD	Mean	SD		
<b>SGPT</b>	44.02	57.33	34.78	18.31	.243	0.808
<b>SGOT</b>	40.97	60.12	27.53	11.45	1.475	0.14
<b>Urea</b>	30.91	5.69	32.84	4.83	1.584	0.113
<b>Creatinine</b>	0.84	0.19	0.83	0.13	.277	0.782
<b>Chol Es</b>	1911.83	1002.65	3220.91	818.91	7.694	<b>&lt;0.001**</b>

SD: Standard Deviation

Chol Es: Choline-esterase

\*\* Highly statistically significant

Table (4) showed that regarding liver and kidney function tests, no significant differences were found between the two groups. Cholinesterase levels showed highly significant differences.

### Discussion

Although million cases of pesticide poisonings are documented every year around the world, data of their cytogenetic effects on occupationally exposed individuals are limited, particularly in developing countries where pesticides have been widely used over the years (Sharma and Sharma, 2012; Sharma et al., 2012).

To investigate this relation, results of the present work revealed that the frequency of the JK-variant in the examined farm workers was 73.8% in heterozygous state with the atypical gene (A) and 13.8% with the usual (U) variant (Table 1) meaning that 87.6% of the affected workers carry the JK-variant which exposed them to the rapid toxicity of OPs.

BChE activity in occupationally exposed workers was low and showed statistically significant difference when compared with the non exposed group (Table 4), which is in agreement with previously reported studies (Hernandez, et al., 2005; Jintana et al., 2009; Howard et al., 2010; Bianco et al., 2017).

Another important finding is that farm workers with the risk genotype (JK- variant) had low levels of cholinesterase activity (Table 2); taking into consideration that the percentage of reduction in activity is the highest in those carrying the JK- variants in the heterozygote state with the atypical (A) gene (AK/JK  $\rightarrow$  49.7%, followed by those carrying U/JK  $\rightarrow$  49.3% then A/JK  $\rightarrow$  43.4%). In the heterozygote state with the A variant, nearly 46% reduction in serum BChE activity was observed, while with the usual variant reduction was 49% (Graph 1) meaning that the A or U doesn't make the change but J and K variants are the responsible. A finding that raises the possibility of synergistic effects between the J and the K variants (neither of them was found alone). In accordance with our study, are the findings reported in 1984 by Evans and Wardell who stated that the J and K phenotypes which are associated

with reduction in measured enzyme activity of approximately 66% and 33% respectively, were unusually identified separately. They can be identified with certainty only when they occur together with the atypical variant.

In comparison with other researchers, Bartels and his colleagues in 1992 examined samples from individuals with K-variant pedigrees. They found that BChE K-variant is one of the most common polymorphism in the BChE gene. It is an alanine-to-threonine substitution in the 539 amino acid position (Ala539Thr)]. They deduced that individuals carrying the K-variant mutation had 30% reduction of serum BChE activity. Also, Shields and Lewis in 2011 examined samples from a small Australian Defense Force and reported that the K-variant is the most common variant in any population and that in vitro enzyme activity studies had demonstrated a 33% reduction in enzyme activity in both heterozygous and homozygous forms.

Similarly, our results showed that the most common variant in the exposed group was the JK variants (87.6%) in the heterozygote state with the A and U variants (Table 1), and that reduction in serum BChE activity was more than

40% in the heterozygote state whether with the A or U variants (Graph 1).

Also, Lockridge and his colleagues in 2016 found that the A and K-variant had only 70% of the standard concentration of BChE molecules per ml plasma and that genetic variant with low levels or with inactive BChE were expected to be at increased risk of toxicity from pesticides.

Bartels and his colleagues in (1992a) identified that the J-variant of human serum (BChE) causes both an approximately two-thirds reduction of circulating enzyme molecules and a corresponding decrease in the level of BChE activity present in serum. They found that the JK variant caused lower BChE activity by about 73% compared to the levels that were produced by the usual variant. Also, Rubinstein and his colleagues in 1978 found 66% decrease in BChE activity in sera from individuals carrying JK variant.

In the present study, no J variant was detected in the non exposed group, the frequency of the K-variant in heterozygous state with the A-variant was 69.1% (Table 1) and 27.3% carried the usual allele in heterozygote state with the A variant. Their pseudo-choline esterase levels (Table 3) were within nor-

mal levels; so large scale studies are needed to identify genetic variations among Egyptian population not exposed to pesticides.

Regarding OPs effects on hepatorenal functions; our results showed that the liver function tests in both groups were slightly higher than normal; in the exposed group [SGPT level (mean 44.02+ 57.33) , SGOT level (40.97+ 60.12) and in the non exposed groups [SGPT level (34.78+ 18.31) , SGOT level (27.53+ 11.45) but with no statistically significant differences between the two groups (Table 4) . The raised levels might be due to other confounding factors.

Kidney function tests were within normal in both groups; with no statistically significant differences between them (Table 4). However, some researches raised the possibility of hepatorenal toxicity in case of chronic exposure due to the deleterious effects of OPs on the liver cytochrome P450 and mitochondria. Nephrotoxic effects are thought to be due to the generation of reactive oxygen species (ROS) leading to renal oxidative stress and toxicity (Khodeary et al.,2009).

### Conclusion and recommendations

This study is considered to be the first reported analysis of common genetic variation in BChE in a sample of farm workers in Egypt. Genetic factors such as choline esterase variants might have played a role in the development of acute and chronic illness and so, genetic monitoring of agricultural workers is greatly recommended.

Safe and proper handling of pesticides during transport, storage, mixing, loading, application and disposal is recommended. Enforcement of existing regulations is needed. All people directly or indirectly involved in the handling of pesticides should be aware of the importance of using appropriate personal protective equipments and the potential hazards of occupational exposure. Adequate training of occupational workers together with the vigorous enforcement of strong pesticide safety regulations and implementation of prevention strategies are greatly recommended.

Future studies should correlate genotype, OP exposure and signs of toxicity.

### Limitations of the study:

1. The small sample size of the examined group limit the ability to deter-

mine the prevalence BChE enzyme genetic variants among Egyptian population although comparison with the non-exposed group gave a picture on genotyping characterization of exposed farm workers.

2. Full data on the type of pesticide used and their amount are unavailable. Similarly, Krenz and colleagues in 2015 found that there were no significant associations between specific pesticides and BChE inhibition. Also, Bull et al. in 2006 stated that it is largely unfeasible to determine the potential effects of any specific pesticide of concern because pesticide products generally comprise a mixture of different chemicals used simultaneously with varying combinations.

### Conflict of interest

None.

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