ASSOCIATION OF GSTM1 AND PARAOXINASE-1 (L55M & Q192R) POLYMORPHISMS WITH CHRONIC ORGANOPHOSPHORUS INTOXICATION IN EGYPTIAN PESTICIDE APPLICATORS

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Submit Date 2022-02-17
Revise Date 2022-10-08
Accept Date 2022-10-15

ABSTRACT

Background: At work, farmers are more likely to come into contact with pesticides and other agricultural contaminants. The permanent inhibition of acetylcholinesterase (AChE) at nerve synapses is thought to be a systemic impact of organophosphates (OPs). However, several investigations have shown that AChE inhibition alone does not account for all toxicological effects associated with long-term OP exposure. Although oxidative stress and epigenetic modifications are two of the most recently hypothesized mechanisms, the existence of genetic variations appears to have the greatest influence on biological outcomes. **Objectives:** The purpose of this study is to investigate the polymorphisms in genes that code for OPs metabolizing enzymes. Because the glutathione-S-transferase (GSTM1) and paraoxonase (PON1) genes encode enzymes that breakdown pesticides with carcinogenic potential, we're particularly interested in them. **Methodology:** From June to December 2018, a cross-sectional study was done on 24 pesticide applicators and 25 corresponding controls. Serum AChE activities were determined using the EQM Test-Mate kit. Using polymerase chain reaction-restriction fragment length polymorphism, the influence of genetic polymorphism in GSTM1 and PON1 on OPs exposure was examined. **Results:** Compared to the control group, chronically exposed patients had significantly lower serum AChE enzyme activity (<0.001). In terms of the GSTM1 polymorphism and the R allele of the PON1 (Q192R) polymorphism, there was a significant difference between chronic OP exposed cases and controls. The PON1 (LM) polymorphism was found to be of little consequence. **Conclusion:** Finally, the GSTM1 and R allele of PON1(LM) polymorphisms may be a risk factor for chronic organophosphates intoxication in Egyptian pesticide applicators.

Keywords: Organophosphates; Acetylcholinesterase; Polymorphism; Glutathione-S-transferase (GSTM1) gene; Paraoxonase (PON1) gene; Polymerase chain reaction-RFLP.

INTRODUCTION

Insecticides that include phosphorus-containing organic compounds are widely used across the world. That is why these compounds represent a great group of chemical weapons, which must be paid attention **(Hreljac and colleagues, 2008)**. People can be exposed to the organophosphorus compounds or their metabolites through several sources, such as the foods and drinks we have daily, as well as through the surrounding environment **(Bevan et al., 2017)**. Three million severe cases of poisoning have been reported in the United States **(Badr, 2020)**. This leads to the death of more than 300,000 people annually **(Satar et al., 2009)**. It is supposed that the inactivation of acetylcholinesterase affects the neurotoxicity of organophosphates after prolonged exposure **(Fryer and colleagues, 2004)**. Researchers indicated that using the anti-cholinesterase alone is not enough to justify many diseases associated with exposure to OPs **(Mbah Ntepe et al., 2020)**. Many toxicological and epidemiological studies have established that OPs may produce oxidative stress by increased formation of O_2 and N_2 free radicals. O_2 and N_2 free radicals interact with biological macromolecules, resulting in physiological changes **(Mbah Ntepe et al., 2020**). Recently, researchers discovered several OP secondary targets **(Hreljac et al., 2008)** that are not associated with the cholinergic system and may lead to disruption of the endocrine system, immunotoxicity, and genotoxicity and carcinogenicity **(Elerek and Filipi, 2011)**. **The International Agency for Research on**

Cancer (**IARC)** has assessed several pesticides as possible human carcinogens **(Teodoro et al., 2019)**. Many pieces of research carried out regarding the side effects of organophosphorus compounds discovered some human disorders, such as neurological, neurobehavioral, reproductive problems as well as malignancies**,** all are caused by chronic exposure to a low dose of OPs (**Duan et al., 2017)**. Therefore, researchers found that the genotoxic tests achieve results more accurately for chronic poisoning and early identification than results achieved by acetylcholinesterase-inhibition tests that are commonly conducted under severe exposure **(Chandrakar et al., 2020)**. The process of carcinogenesis develops through three phases: initiation, promotion, and progression. Carcinogens that are non-genotoxic primarily cause the transformation to malignant cells and enhance the proliferation of cells in many aspects, comprising avoiding apoptosis, augmenting growth factors, and escaping growth suppressive signals **(Salem et al., 2020)**. Organophosphates undergo biotransformation mainly in the liver by cytochrome P450s 3A4 and 3A5 (Phase I enzymes**)** where an active intermediate organophosphorus-oxon is produced **(Kapka-Skrzypczak et al., 2011)**. Paraoxonase enzyme subsequently hydrolyzed this active form of organophosphorus-oxon to produce diethyl phosphate and 4-nitrophenol, or bound to glutathione, by glutathione S-transferases catalyzing the reaction **(Singh et al., 2011)**. In humans, the following genotypes of the CYP3A5, PON1, PON2, GSTM1, GSTT1, and GSTP1 genes have been discovered **(Liu et al., 2006)**. Some disorders, such as premature birth, have been taken into consideration due to their impact on disease vulnerability, such as DNA damage and tumorigenesis **(Abdel Hamid et al., 2017)**. Human susceptibility to OP toxicity is assumed to be predisposed by PON1 polymorphisms **(Naksen et al., 2015)**. The human paraoxonase 1 enzyme (PON1) is linked with the high-density lipoprotein (HDL) that has broad substrate specificity. PON1 is an anti-inflammatory and antioxidant enzyme serving as a paraoxonase and arylesterase **(Yildiz et al., 2017)**. The single nucleotide polymorphisms (SNPs) in the coding regions (192Q/R and 55LM) of the PON1 gene have been found. PON1 activity and levels are influenced by the L55M **(Ceja-Gálvez et al., 2020)**. The Q192R polymorphism influences the enzyme's hydrolytic activity **(Yildiz et al., 2017)**. Due to genetic variation in GSTs, the GSTM1 gene has polymorphism, and its null genotype may impact reducing enzyme activity. Therefore, the said changes increase the risk of carcinogenesis **(Ratna et al., 2020)**. The evaluation conducted on these chemical compounds in terms of genotoxicity must be maintained and expanded **(Cakir and Sarikaya, 2005)**.

SUBJECTS & METHODS

Study population

Between June and December of 2018, a cross-sectional investigation was carried out. There were 49 male volunteers in the study, who were split into two groups. There were 24 applicators of pesticides in the study group. All of them were working in this profession three years ago at least. Our control group consisted of 25 males from rural areas who had the same age as the experimental group, but they were not vulnerable to organophosphates exposure at the workplace. Both groups had to be between the ages of 18 and 60, and they couldn't have a history of chronic sickness or severe pesticide toxicity. All the subjects were submitted to a medical history, physical assessment, and lab tests. Medical records (any previous or present diseases or medicines), individual history (age, place of residence, and tobacco use), and work experience are all factors to consider (duration of work). After all engaged individuals were informed about the study's goal and methodology, Menoufia University's Faculty of Medicine's Ethical Committee revised and approved the study. The original Ellman technique was utilized to test serum AChE by using the EQM Test-Mate kit (EQM Research Inc., Cincinnati, OH, USA) **(Ellman et al., 1961).**

DNA extraction for genotyping

Venipuncture was performed to retrieve 5 mL of venous blood, which was then deposited into sterile vacutainers containing EDTA and maintained at (-20°C). DNA extraction and GSTM1 and PON1 polymorphism gene analyses were performed. In agreement with the instructions of the manufacturer, the DNA was isolated from whole blood from peripheral circulation by using a DNA extraction kit (iNtRON Biotechnology's G-spinTM Mini Kit for Total DNA Extraction). Extracted DNA was kept at -20°C till it was needed.

GSTM1 Genotyping

GSTM1 was amplified using restriction

fragment length polymorphism in the polymerase chain reaction (PCR-RFLP). Each 25 µl reaction mixture contained 12.5 µl DreamTaq PCR 2X Master Mix (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States) and 20 pmol of GSTM1 (5′-GAA CTC CCT GAA AAG CTA AGC-3′ and 5′-GTT GGG CTC AAA TAT ACG GTG G-3′). After a 5-minute denaturation phase at 94°C, 35 cycles of 1 minute at 94°C, 1 minute at 58°C, 1 minute at 72°C, and a final polymerization phase of 10 minutes at 72°C were performed. The products that had been amplified were examined using electrophoresis on 2 percent agarose gels stained with ethidium bromide. A band's presence or absence indicated wild or deletion genotypes in 215 GSTM1 genotypes (**Fig. 1)**.

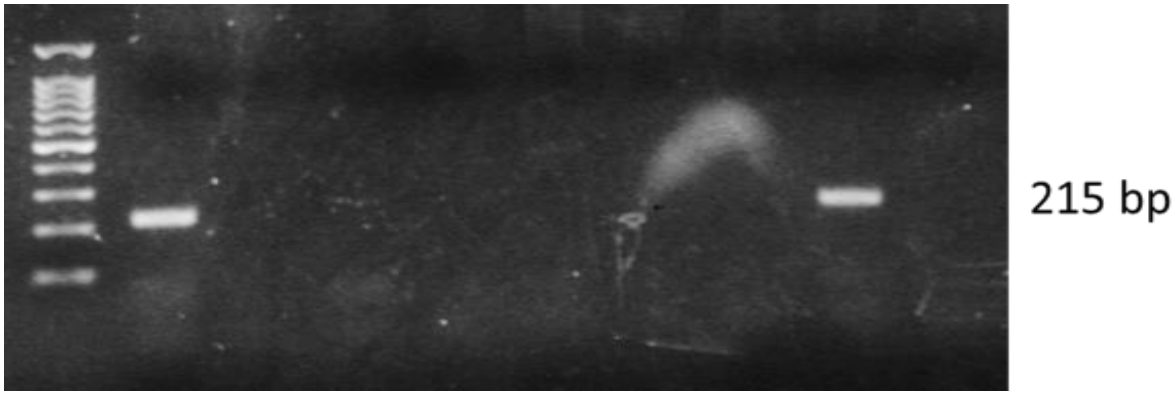

Figure (1): Electrophoretic analysis of GSTM1 polymorphism in 2% agarose gel. 215 bp indicated the wild existence genotype of GSTM1.

PON1 (L55M) genotyping

The Paraoxonase-1L55M gene was genotyped using RFLP-PCR. In a final 25- µl volume, PCR was performed with 10 µl of DNA template and a master mix of 12.5 µl [DreamTaq PCR 2X Master Mix (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States), 1 µl of forward primer 50 mmol/l (5'CCT GCA ATA ATA TGA AACAAC3'), 1 µl of reverse primer 50 mmol/l (5'-TGA AAG ACT TAAACTGCC AGTC-3')], and 0.5 µl of distilled water. Applied Biosystems performed the PCR amplification (Foster City, California). After denaturation at 94°C for 6 minutes, 32 cycles were performed (1 minute of denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of extension at 72°C). The last extension step was then done for 4 minutes at 72°C. NlaIII (New England Biolabs, Ipswich, Massachusetts, USA) was used to digest the PCR product at 37°C for 1 hour. (2.5 µl of 10 NE buffer 4, 1 µl of NlaIII, 6.5 µl of distilled water, and 10 µl of PCR product). On 3 percent agarose gel, NlaIII digestion products were electrophoresed for 30 minutes. It was stained with ethidium bromide. Ultraviolet light was used to see the bands. PCR products digested with the L allele were 172 bp, while those digested with the M allele were 106 and 66 bp **(Fig. 2)**.

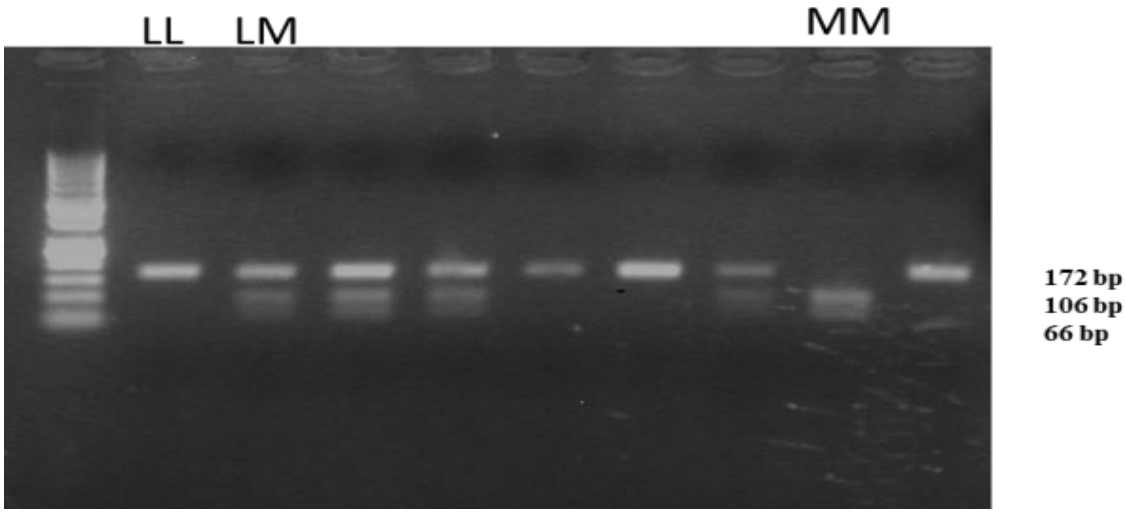

Figure (2): Electrophoretic analysis of restriction fragments of PON1 (LM) gene plymorphism in 3% agarose gel. LL genotype in lines 1, 5, 6; LM genotype in lines 2,3,4, while MM genotype in line 8

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PON1 Q192R genotyping

The genotypes of PON1 Q192R were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test.

The following primers were used: The primers that followed amplified a 99-bp band. R: 5'-CAC GCT AAA CCC AAA TAC ATC TC -3' and F: 5'-TAT TGT TGCTGT GGG ACC TGA G-3'. 3µl genomic DNA, 12.5 µl DreamTaq PCR 2X Master Mix (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), and 7.5 µl sterilized distilled water was used in a total volume of 25 µl for PCR. A polymerase chain reaction was performed on PON1 Q192R, which began with a 5-minute denaturation at 94°C. For amplification, there were 35 cycles of 1 minute at 94°C, 1 minute at 59°C, and 1 minute at 72°C. The reaction was finished after another 7 minutes of extension at 72°C. PCR results were examined further utilizing standard RFLP. PON1 Q192R was made with the help of the BspP1 enzyme. Utilizing a Promega gel electrophoresis apparatus and UV transillumination, the digest was run on 3 percent agarose gel with ethidium bromide labeling. The restriction enzyme did not digest the QQ genotype, yielding a 99-bp product, but it did digest the RR genotype, resulting in two 66-bp and 33-bp fragment bands in PON1 Q192R. In the QR heterozygote pattern, 99 bp, 66 bp, and 33 bp components were discovered.

Statistical analysis

The data will be assembled, tabularized, and statistically analyzed using IBM SPSS statistics software for Windows, version 22.0. (Chicago, IL, USA). Quantitative data were given as mean and standard deviation (SD), whereas qualitative values were presented as percentages. The independent sample t-test or Mann–Whitney test was used for quantitative data, whereas the Chi-square (χ^2) test was utilized for qualitative variables. A p-value of less than 0.05 was judged statistically significant.

RESULTS

The mean age was 38.3 ± 12.99 and 37.95 ± 11.36 years for the control and subjected groups respectively. Both groups were coordinated regarding age ($p > 0.05$). No distinction was detected between the two groups in terms of smoking behavior; however, the distribution among the subjected group was significantly different where smokers represented 83.3%. Furthermore, no significant differences were across both groups as regards body mass index (BMI) and learning. AChE enzyme activity in serum was significantly inhibited in subjects with long-term exposure as opposed to the control group **(Table 1).**

Table 1: Characteristics and serum acetyl cholinesterase activity among the exposed and the control groups.

Variables	Exposed (n=24) Mean± SD	Controls (n=25) Mean± SD	Chi-square (χ^2) test	p-value
Age (years)	38.30±12.99	37.95±11.36	U=0.10	0.959
Education:			χ^2 =1.93	0.380
Illiterate	14 (58.3)	12 (48.0)		
Read & write	9 (37.5)	9 (36.0)		
Secondary education	1 (4.2)	4 (16.0)		
Smoking:				
Smokers	20 (83.3)	18 (72.0)	χ^2 =0.01	0.840
Non-smokers	4 (16.7)	7 (28.0)		
BMI (kg/m^2)	27.62 ±2.24	27.89 ±2.58	t=0.40	0.691
AChE (U/ml)	87.34±13.53	108.25±24.61	t=3.82	<0.001*
Duration of work (years)	10.48±9.66			

***Statistically significant at p < 0.05 χ^2 = Chi square test**

Molecular genotyping: The genotype and allelic distribution of GSTM1 wild and null genotypes were presented in **fig. (1)** and **table (2).**

Table (2): Comparison between the studied groups regarding prevalence of GSTM1 mutant genes

Item	Exposed group (n=24) No (%)	Control group (n=25) No (%)	Chi-square (χ^2) test	p-value
GSTM1:				
Wild type (M1)	5 (20.8)	12 (48.0)	3.99`	0.046*
Deletion type	19 (79.2)	13 (52.0)		

***Statistically significant at p < 0.05 χ^2 = Chi-square test**

GSTM1 null genotype was present in 79.2% of exposed subjects and present in 52% in control and it differs significantly between the subjected cases and control. Concerning the Q192R polymorphism; in homozygotes for the Q192 allele, an undigested fragment (238 bp) was discovered (genotype QQ) but in homozygotes for the R192 allele digested fragments (175 and 63 bp) were found (genotype RR). In heterozygotes, the digested and the undigested fragments (238, 175, and 63bp) were found (genotype QR). In terms of genotype and allele frequency of PON1, no statistically significant difference was found regarding the PON1 LM polymorphism between both groups tested. The distribution of PON1 genotype LL, genotype was existing in 40% of controls and 45.8% of subjected cases. LM genotype was existing in 48.8% and 45% of control and case groups respectively. MM genotype was 12% in control and 8.3% in subjected cases. The incidences of L and M alleles were 68.8 and 31.2% in subjected cases and 64 and 36% in controls, correspondingly **(Table 3)**.

Table (3): Comparison between the studied groups regarding PON1 genotypes and alleles at L55M.

Item	Exposed group (n=24) No (%)	Control group(n=25) No (%)	Chi-square (χ^2) test	p-value
L55M:				
LL	11 (45.8)	10 (40.0)		
LM	11 (45.8)	12 (48.0)	0.27	0.783
MM	2 (8.3)	3 (12.0)		
	N=48	N= 50		
L allele	33 (68.8)	32 (64.0)	0.25	0.619
M allele	15 (31.2)	18 (36.0)		

Statistically non-significant at p >0.05 χ^2 = Chi square test

Pertaining to the resultant bands, PCR fragment size and relation to genotyping are displayed in **Fig. (3)** and **Table (4)**.

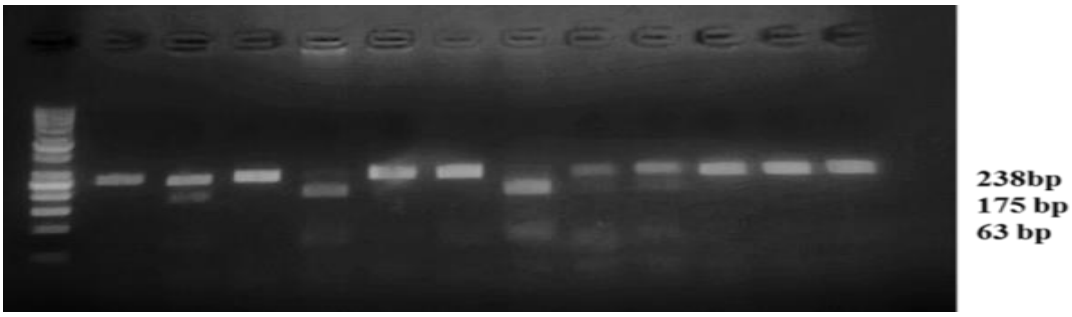

Figure (3): Electrophoretic analysis of restriction fragments of PON1 (Q192R) gene polymorphism in 3% agarose gel. QQ genotype in lines 1, 3, 5, 6; QR genotype in lines 2,8,9, while RR genotype in line 4, 7.

Table (4): Comparison between the studied groups regarding PON1 genotypes and alleles at Q192R.

Item	Exposed group (n=24) No (%)	Control group (n=25) No (%)	Chi-square (χ^2) test	p-value
Q192R:				
QQ	13 (54.2)	20 (80.0)		
QR	7 (29.2)	5 (20.0)	5.80	0.055
RR	4 (16.7)	0		
	N=48	N= 50		
Q allele	33 (68.8)	45 (90.0)		
R allele	15 (31.2)	5 (10.0)	6.81	0.009*

***Statistically significant at <0.01 χ^2= Chi square test**

Table (4) shows the genotype frequency of PON1 Q192R; homozygous (QQ) had been the most prevalent genotype in control subjects, thereafter heterozygous (QR) and homozygous mutant gene (RR), their frequencies were (80%), (20%) and (0%), correspondingly. The most prevalent genotype in patients was homozygous (QQ) [(54.2%)], followed by heterozygous (QR) [(29.2%)] then the homozygous mutated gene (RR) (16.7%)]. The incidence of the Q allele was found to be (68.2%) and (90%), while that of the mutated R allele was (31.8%) and (10%) in the patients and control group, correspondingly. Comparing the treated and control groups, no significant difference was detected in the distribution of PON1 Q192R genotypes (p=0.055). However, the frequency distribution of the Q and R alleles was significantly different between subjected and control groups (p=0.009) **(Table 4)**.

DISCUSSION

Pesticide poisoning has been linked to a large number of deaths and illnesses, according to research. Pesticides containing OPs account for more than 80% of all pesticides used worldwide. Pesticides are generally administered in groups rather than separately in agricultural operations **(Mbah Ntepe et al., 2021)**. Because of the likelihood of a synergistic impact of two OPs, even a modest non-lethal dosage of a mixture of OPs may offer an increased hazard **(Abdel Hamid et al., 2017)**. Exposure to OPs has been associated with cancer in studies, with over 14 million novel cases reported in 2012 **(Pluth et al., 2019)**. Statistically significant inhibition of RBC-AChE was detected in OP exposed (87.3413.53) versus control (108.2524.61), which is regarded as proof of OPs exposure. Previous investigations have found that a decrease in AChE activity is a key component and an important biological marker of OPs exposure **(Mbah Ntepe et al., 2021)**. The most often found polymorphisms of cytochrome P450, glutathione transferases (including GSTM1, GSTP1, GSTT1), acetyltransferases, and paraoxonases (mainly PON1), which are all engaged in the metabolism of OP, can be used to assess susceptibility to exposure. **(Kapka-Skrzypczak et al., 2011)**. Many researchers have directed their attention to the GSTs and paraoxonase (PON1, 2, 3) families recently and updated literature data constantly **(Teodoro et**

al., 2019). GSHs (GSTP1, GSTT1, and GSTM1) are a family of enzymes that deactivate free oxygen radicals, precluding them from interacting with other proteins and enzymes essential for cellular function **Mbah Ntepe et al., 2020)**. The impact of genetic polymorphism on the metabolic pathways of OPs is more emphasized and reviewed with their individual alternatives which may explain the exposure to various toxins **(Teodoro et al., 2019)**. Due to the genetic variation in GSTs, the genotype of GSTM1 null may reduce the enzyme activity. Changes in the enzyme activity provoke a chance for carcinogenesis **(Ratna et al., 2020)**. The GSTM1 null genotype was found in 79.2 percent of subjected cases and 52 percent of controls, with a significant difference between the subjected and control cases **(table 2)**, suggesting that OPs applicators may be more susceptible to illness. Some carcinogens cannot be detoxified via the glutathione-conjugation pathway by people who have GSTM1 gene deletion **(Singh et al., 2012).** GSH is an antioxidant, meaning it protects the cell against free radicals. GSH levels were shown to be reduced after exposure to a mixture of pesticides, indicating the creation of Reactive Oxygen Species (ROS), and additional GSH was used by associated enzymes to neutralize ROS effects **(Ratna et al., 2020)**. More severe DNA damage in leukocytes in the peripheral circulation may result from increased ROS paired with lower GST activity. DNA damage is more likely in those with GSTM1 null genes. Higher DNA adduct production and sperm DNA damage have also been linked to the GSTM1 null genotype in males who are subjected to polycyclic aromatic hydrocarbons on the job. In pesticide-exposed employees, With the PON1 Gln–Gln genotype, Da Silva et al found a higher frequency of micronuclei in GSTT1 and GSTM1 positive genotypes. Individuals missing GSTM1 and GSTT1 genes were presented to be more susceptible to tobacco smoking-related genotoxicity, as stated by Norppa **(Singh et al., 2012)**. In contrast, Kumar et al revealed that positive people had damage at higher levels for both GSTT1 and GSTM1 significantly; furthermore, persons with positive GSTM1 had immunosuppression, as the NK cells percent recognized in these individuals was considerably low. An increase in genetic damage (MNL) was also discovered by Falck et al. among GSTM1-positive greenhouse workers exposed to pesticides **(Kumar et al., 2016)**. As a result of these discrepancies, some researchers advised against using GST polymorphisms to predict sickness risk related to pesticide exposure **(Costa et al., 2014)**. Many research papers recovered a link between GSTM1 null genotypes and increased possibility of cancer, involving the urinary bladder, lung, and colorectal cancers. Although previous research has studied the modulatory effect of GST polymorphism, notably the null genotypes GSTM1/GSTT1, on DNA damage coupled with exposure to pesticides **(Singh et al., 2012)**, the outcomes are restricted and conflicting **(Tumer et al., 2016)**. Workers who have GSTP1Ile/Ile wild genotype combined with either GSTM1null genotype or simultaneous deletion of GSTT1/GSTM1 genotype had an abundant higher MN frequency than workers with wild type/functional combinations of enzymes. These results further underline the value of the GSTM1 isozyme in determining the DNA damage susceptibility of an individual because of pesticide exposure. Overall, Tumer et al. **(Tumer et al., 2016)** study found a link between the GSTM1 null genotype and a higher frequency of MN, suggesting that GSTM1 is possibly one of the most important predictors of genotoxic risk in individuals who are exposed to pesticides, thus can be a successful susceptibility biomarker candidate in humans biosurveillance studies **(Tumer et al., 2016)**. Following activation by cytochrome P450 in the liver, organophosphates are hydrolyzed by PON1. PON1 polymorphism can modify the effectiveness of the response to damage of DNA in organophosphate pesticide applicators **(Teodoro et al 2019)**. The PON1*192Q and PON1*55L alleles are considered the wild types of these two polymorphic locations as they are the types that are dominant in some people. On codon 192, single-nucleotide polymorphism (SNP), gives rise to the amino acid substitutions of arginine (R) for glutamine (Q). The result of this mutation is the*192R allele. Nucleotide substitution on codon 55 results in methionine being substituted for leucine. The *55M allele is the result of this mutation **(Ginsberg et al., 2009)**. The distribution of genotype PON1 Q192R was not significantly different between both control and treated groups (p=0.055). A significant difference in the R allele of PON1 (Q192R) polymorphism was detected between controls and chronic OPs exposed subjects

[(p=0.009) **Table (4)**]. One of the two PON1 alloforms is better at metabolizing particular OPs than the other. Paraoxon is hydrolyzed faster by the PON1 192RR isoform than by the PON1 192QQ isoform, on the other hand, the PON1 192QQ isoform, hydrolyzes diazoxon sooner than the PON1 192RR. The existence of the R allele correlates to the gene-dependent increase in PON1 activity, proposing a biosensor effect through exposure to an anticholinesterase agent that may overexpress PON1 activity **(Ratna et al., 2020)**. The PON1 192Q and 55L polymorphisms were displayed to boost the risk of organophosphate toxicity in the meta-analysis. Additional ethnic subgroup research revealed that the PON1 192Q and 55L polymorphisms are linked to a high possibility of organophosphate poisoning in Caucasians. Similar correlations, alternatively, were not observed among Asian populations. Ultimately, the existing meta-analysis proposes that polymorphisms of PON1 192Q and 55LM may enhance the hazard of organophosphate poisoning, particularly in Caucasian populations **(You et al., 2013)**. Hreljac et al discovered that OP methyl parathion was more genotoxic than its metabolite methyl paraoxon in HepG2 cells. This denotes that AChE is not included in methyl parathion and methyl paraoxon genotoxicity, but other processes are concerned. Dimefox, an extremely poisonous OP, did not cause breaks in the DNA strand but did exhibit action that is mitogenic in the same research. In terms of carcinogenicity, according to Reuber, dimethoate and its poisonous metabolite, omethoate, can generate benign and malignant hepatic neoplasms. **IARC**, on the other hand, was incapable to encounter sufficient evidence to entitle dimethoate as a possible carcinogen. In addition, research by Bonner et al found no link between malathion and malignancy in pesticide applicators. All these data denote that agent that cause significant DNA and cell damage are mutagenic and/or carcinogenic but those that cause only a little DNA damage are not mutagenic or carcinogenic **(Karami-Mohajeri et al., 2017)**. In occupationally exposed people, several cytogenetic tests have been employed to evaluate the probable pesticide-related genotoxicity. However, there have been registers of both positive and negative genotoxic influences in pesticide-exposed populations **(Liu et al., 2006)**. Individual responses and genetic predispositions, as well as variations in sensitivity to a certain chemical, are key issues in pesticide exposure studies at work. Pesticide toxicity and increased or decreased susceptibility to certain chemicals may also be influenced by pesticide-metabolizing enzyme genetic polymorphisms (SNPs) **(Mbah Ntepe et al., 2020)**. Most of the research on DNA damage and pesticide exposure has been dedicated to cytogenetic endpoints including CA, SCE, and MN, with mixed outcomes. Some display a considerable rise in the frequency of MN, SCE, and CA, while others display no significant changes. There is also a great deal of uncertainty encircling exposure to pesticide and genotoxic impacts research, such as the fidelity of exposure estimation, the study's power, the adequacy of control groups, and the genotoxicity procedures utilized **(Kapka-Skrzypczak et al., 2011)**. Consequently, discrepancies across research may be enlightened by the multiplicity of pesticides used in many parts of the world where agriculture is practiced.

CONCLUSION

The introductory study indicated a significant difference between chronic OP exposed cases and controls concerning the GSTM1 polymorphism and R allele of the PON1 (Q192R) polymorphism, while no significant difference was observed regarding the PON1 (LM) polymorphism. Finally, the GSTM1 and PON1 polymorphism R allele may be risk factors for chronic OP intoxication in Egyptian pesticide applicators.

RECOMMENDATION

Because decision-making on polymorphic research necessitates big sample sizes and studies in several groups and societies, investigations with greater sample sizes on GSTM1 and PON1 polymorphisms in our population and others are recommended. Follow-up is of possible benefit for (the GSTM1 polymorphism and/or R allele of the PON1 polymorphism) organophosphorus pesticides' applicators.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there was no conflict of interest.

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تعدد النمط الچيني لإنزيمي الجلوتاثيون-S-ترانسفيراز -M1والباروكسوناز-1 مع التعرض المزمن فى المصريين العاملين برش المبيدات العضوية الفوسفورية

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مقدمة:

يتعرض الفلاحون لخطر متزايد من الآثار السامة بالتعرض لفترات طويلة لرش المبيدات العضوية الفوسفاتية والمواد الكيميائية الزراعية الأخرى. يُعتقد أن التأثيرات الضارة للفوسفات العضوي على صحة الأنسان لها تأثير جهازي، أي تثبيط لا رجعة فيه لإنزيم أسيتيل كولين إستيراز في الموصلات العصبية. ومع ذلك، فقد أظهرت العديد من الدراسات أن تثبيط إنزيم الأسيتيل كولين إستيراز وحده لا يمكن أن يفسر جميع المظاهر السمية بالتعرض لفترات طويلة للرش بتلك المبيدات .بعض الآليات المفترضة مؤخرا يمكن أن تعزى لوطأة الأكسدة والتعديلات فوق الجينية، ولكن يبدو أن التأثيرات البيولوجية يتم تعديلها بشكل أساسي من خلال حدوث تعدد النمط الجيني .

الهدف من البحث:

تهدف هذه الدراسة إلى تقييم تأثير تعدد النمط الجيني لكل من چين إنزيم الجلوتاثيون-S-ترانسفيراز -M1والباروكسوناز-1 على المشتغلين برش المبيدات العضوية الفوسفاتية مع احتمالية التسبب في حدوث السرطان وذلك نظرا لأن كلا منهما يحمل الشفرة الجينية للإنزيمات التي تستقلب المبيدات العضوية الفوسفاتية .

أشخاص وطرق البحث:

أجريت هذه الدراسة المقطعية على 24 من المصريين المشتغلين برش المبيدات و25من الأصحاء غير المشتغلين كمجموعة ضابطة .تم قياس مستوى إنزيم الأسيتيل كولين إستيراز .وباستخدام تفاعل البلمرة المتسلسل تم فحص تعدد النمط الجيني لكل من چين إنزيم الجلوتاثيون-S-ترانسفيراز -M1وچين الباروكسوناز-1.

النتائج:

لوحظ وجود انخفاض ذو دلالة إحصائية في مستوى إنزيم الأسيتيل كولين إستيراز في المشتغلين برش المبيدات بالمقارنة بالمجموعة الضابطة .وباستخدام تفاعل البلمرة المتسلسل كان هناك ازدياد ذو دلالة إحصائية بين حالات المشتغلين برش المبيدات بالمقارنة بالمجموعة الضابطة فيما يتعلق بتعدد النمط الچينى فى چين إنزيم الجلوتاثيون-S-ترانسفيراز -M1والباروكسوناز-1 الأليل R. بينما لم نلاحظ فرقا ذو دلالة إحصائية فيما يتعلق بتعدد النمط الچينى لـلباروكسوناز-1 (LM) .

الاستنتاجات والتوصيات:

قد يمثل تعدد النمط لچين إنزيم الجلوتاثيون-S-ترانسفيراز -M1ونمط چين إنزيم الباروكسوناز-1 الأليل R عامل خطورة في المشتغلين برش المبيدات العضوية الفوسفاتية وذلك بحدوث مضاعفات كالسرطان. المتابعة ذات فائدة محتملة لمستخدمي مبيدات الآفات العضوية الفوسفات (تعدد النمط لچين إنزيم الجلوتاثيون-S-ترانسفيراز-M1 و نمط چين إنزيم الباروكسوناز-1 الأليل