GENETIC POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASE (M1 AND T1) AND PARAOXONASE 1 (PON1) AND SUSCEPTIBILITY TO CHRONIC KIDNEY DISEASE OF UNKNOWN ETIOLOGY IN PESTICIDE EXPOSED PATIENTS AT ZAGAZIG UNIVERSITY HOSPITALS

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ABSTRACT

Background: Pesticide compounds are commonly used worldwide and in Egypt. Chronic low level exposure of humans to these compounds has resulted in many health adverse effects. There are many reports relating chronic kidney disease of unknown etiology (CKDu) epidemic to pesticide exposure including organophosphates (OP). Glutathione-S-transferase mu (GSTM1) and glutathione-S-transferase theta (GSTT1) are phase II xenobiotic metabolizing enzymes. Paraxonase 1 (PON1) is an enzyme linked to high-density lipoprotein. It can prevent oxidation of low-density lipoproteins and participates in organophosphate detoxification. **Objective**: To investigate the association of genetic polymorphism of GST (M1, T1) and PON1 Q192R with CKDu in pesticide exposed patients presented to Nephrology Unit, Internal Medicine Department, Zagazig University Hospitals, Sharkia Governorate. **Methods:** Gas chromatography- mass spectrometry (GC-MS) was used for detection of dialkyl phosphate metabolites in urine samples of subjects included in the study then genomic DNA isolated from the blood samples of 48 CKDu patient chronically exposed to pesticides giving positive result for dialkyl phosphate metabolites and matched control group was used to detect the presence or absence of the GSTM1 and GSTT1 genes (gene deletion) by multiplex polymerase chain reaction technique (multiplex PCR) and PON1 polymorphisms using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. **Conclusion**: This study suggested that there is a correlation between chronic exposure to organophosphates and the risk of CKDu due to GSTM1 and GSTT1 genes deletions and PON1 gene polymorphisms.

Keywords: Glutathin S transferase, Paraoxonase, Chronic kidney disease of unknown etiology, Polymorphism

INTRODUCTION

Chronic kidney disease of unknown etiology (CKDu) represents about 10% of CKD patients globally **(Siddarth et al., 2015)**. An elevated prevalence of CKDu in agricultural communities, predominantly among male farmworkers has been reported in Central America, Egypt, India and Sri Lanka **(Almaguer et al., 2014).** In Egypt, the cause of end stage renal disease (ESRD) is unknown in 27% of patients **(Kamell and El-Minshawy, 2010 & El-Minshawy, 2011a).**

Diabetes and hypertension constitute the major risk elements for chronic kidney

disease (CKD) worldwide. In low income countries, glomerulonephritis is also important **(Barsoum, 2006).** Nevertheless, in the last two decades, a severe type of CKD has been defined in patients without these risk factors. CKD of unknown etiology (CKDu) involves adults in the third to fifth decade of life and is frequently lethal due to disease progress in addition to deficiency of dialysis or transplantation in the concerned geographic regions **(Weaver et al., 2015).**

Though the triggers of CKDu have not been settled conclusively, there is growing evidence incriminating agricultural compounds **(Jayasinghe, 2014).** A case control study of ESRD Egyptian patients established a correlation with rural residence and pesticide exposure **(El-Minshawy, 2011b)**

Efforts to decrease pesticide exposure through the use of further technologies to control pests and organic agricultural practices continue, however exposure to pesticides occupationally and through residue in domestic dust, and in foodstuff and water is widespread **(Michael and Alavanja, 2009).**

In Egypt, where agricultural activities account for 28% of total national income, great risks of pesticide exposure was found due to incorrect application techniques, the use of toxic chemicals which are banned or restricted in other areas, inadequate storage practices, inadequately maintained or totally improper spraying equipment and even the reuse of old pesticide bottles for storing food and water **(Ibitayo, 2006).** EL-Sharqia governorate is considered an agricultural community where organophosphate is the most common type of pesticide used **(Isawi, 2012).**

There are several previous reports of organophosphate induced nephrotoxicity, Malathion induced immune-complex nephropathy has been reported by **Albright (1984).** In addition, **Wedin (1992)** suggested that Malathion possibly have direct toxic effect on renal tubules and they can alter renal function significantly due to alteration in neural, humoral and metabolic activity. **Peiris-John et al. (2006)** also suggests a possible association between CRFu, acetycholine esterase levels and long term low-level exposure to organophosphates.

The metabolism of most OP compounds yields six terminal products that are excreted in urine; they are termed dialkylphosphates (DAP). They involve dimethyl group including: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), and diethyl group including: diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP) **(Wessels et al., 2003).** Urinary DAP metabolites give valuable data about accumulative exposure to OP pesticides **(Barr et al., 2004).** They are currently used as a biomarker of human exposure to organophosphorus insecticides **(Forsberg et al., 2011).**

Closely related to biomarkers of exposure are the biomarkers of susceptibility which determine the individual responses to environmental toxicants and vulnerability to toxicant-induced diseases **(Au et al., 1999).**

Organophosphates are primarily metabolized in the liver through hepatic cytochrome P450 3A4 and 3A5 (Phase I enzyme) to produce the active intermediate organophosphorus-oxon. Phase II metabolizing enzymes include glutathione S-transferases (GST) which are considered a major group of detoxifying enzymes **(Wong et al., 2006).** In addition, organophosphorus-oxon may

then be hydrolyzed by paraoxonase (PON) to diethyl phosphate (DEP) and 4-nitrophenol, or conjugated to glutathione (GSH) **(Barr et al., 2004).**

The polymorphic genotypes of the enzymes GST and paraoxonase-1 involved in the metabolism and detoxification of pesticides including organophosphates and their impact on disease susceptibility were studied for certain disorders as parkinsonism **(Narayan et al., 3013),** preterm delivery **(Banerjee et al., 2014)** and DNA damage and cancer **(Kapka-Skrzypczak et al., 2011)**, however their association with CKDu is rarely studied.

THE AIM OF THE STUDY

To investigate the association of genetic polymorphism of GST (M1, T1) and PON1 Q192R with CKDu in pesticide exposed patients presented to Nephrology Unit, Internal Medicine Department, Zagazig University Hospitals, Sharkia Governorate.

SUBJECTS AND METHODS

This work was conducted through a case control study. The design of the study was approved by the Ethical Committee of Faculty of Medicine, Zagazig University. All subjects were of Egyptian nationality. After explanation of the research objectives and procedures and ensuring complete confidentiality of data, a signed written informed consent was obtained from each subject before participation.

Cases of chronic kidney disease, defined by criteria described by Kidney Disease Improving Global Outcomes **(KDIGO, 2013)** who were admitted to Nephrology Unit of Internal Medicine Department, Faculty of Medicine, Zagazig University during period from June 2015 to December 2016 (total number 400 cases) were asked to answer a questionnaire that include demographic data (age, sex, residence), occupation, history of any medical disease.

Exclusion criteria: Diabetes, hypertension, any previous renal disease or infection, previous operations, exposure to snake bite, medications used **(appendix 1)**.

Out of the four hundred cases of chronic kidney disease, one hundred and ten cases were found to meet the criteria of CKDu. CKDu was identified based on the criteria settled by the Scientific Committee of the National Research Program for CKDu commenced by World Health Organization in collaboration with the Ministry of Healthcare and Nutrition in Sri Lanka **(Research program for CKDu in Sri Lanka, 2009).**

Cases of CKDu occupationally exposed to pesticide and not yet on dialysis were the target of our study (58 cases). They were subjected to urine sample in order to detect organophosphorus metabolites which considered as valuable data about accumulative exposure to organophosphorus (identify exposure), then subjected to blood sample for assessment and investigation of genetic polymorphism of GST (M1, T1) and PON1 Q192R with CKDu in pesticide exposed patients.

A similar number of age, sex and occupation matched healthy subjects were recruited as a control group. Control group was chosen from healthy individuals coming with the patients and hospital team putting in mind not to involve direct relatives of the patients. Age matching was done in 5 years intervals.

I- Detection of organophosphorus metabolites in urine:

For each subject included in the study 10 ml of first morning void urine was collected in a polypropylene container **(Kissel et al., 2005)**. The containers were prewashed in 10% nitric acid for more than 3 hours and then rinsed twice in purified water. The samples were stored at -20ºC for organophosphorus metabolite detection. According to **Tarbah et al., (2004)**, DAP metabolites are stable under storage at -20 ºC. Preparation was performed according to the method described by **Hardt and Angerer (2000).** Briefly, before analysis urine was thawed and the internal standard was added. The internal standard was prepared by dissolving 50 mg dibutylphosphate in 50 mL methanol then diluted with water to produce a concentration of 10 mg/L. Then, HCL was used for acidification followed by two cycles of liquid/liquid extraction with 1:1 mixture of diethyl ether and acetonitrile. The organic layer was vaporized until desiccation and the deposit was thawed in dehydrated acetonitrile. The DAP metabolites were derivatized using pentafluorobenzyl bromide (PFBBr). Water and hexane were added and the DAPs were shifted to the hexane portion by shaking the tubes. The hexane portion was concentrated and analyzed with GC-MS.

The GC-MS analysis was performed in Central Lab of the National Research Centre, El-Dokki, Cairo. Helium was used as a carrier gas with splitless injection of 1µL. The temperature programming was as follows: injection port temperature was 260ºC; column 90ºC for 1 min, then raised at a rate 25ºC/min to 120ºC and maintained for 2 minutes, raised at 6ºC/min to 180ºC and maintained for 25 minutes than raised at a rate of 10ºC/min to 250ºC and isothermal for 34 min; transfer line temperature 300ºC. Mass spectrometer was operated in electron impact (EI) ionization with electron energy of 70 eV; multiplier 2300 V; selected ion monitoring (SIM) mode.

II- DNA extraction:

For each participant, two milliliter of venous blood was collected in EDTA-treated tubes analysis and stored at -20°C until analysis. The samples were coded the analysis was blind. Genomic DNA extraction was performed using FavorPrep Blood Genomic DNA Extraction Mini Kit (Cat.No. FABGK 001) from Favorgen Biotech Corp.; following the manufacturer's instructions.

III- Genetic analysis:

GSTT1 and GSTM1 genes deletions detection:

GSTT1 and GSTM1 genes deletions was verified using multiplex polymerase chain reaction (PCR) according to the technique described by **Arand et al., (1996)**. The primer pairs for GSTT1 gene was: forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and reverse 5'-TCA CCG GAT CAT GGC CAG CC-3', while the primer pairs for GSTM1 gene was: forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and reverse 5'-GTT GGG CTC AAA TATA CGG TGG-3'. The internal control used was albumin gene with forward 5' –GCC CTC TGC TAA CAA GTC CTA C-3' and reverse 5'-GCC CTA AAA AGA AAA TCG CCA ATC-3'. The protocol of amplification was one cycle 95ºC for 2 minutes then 30 cycles of 94ºC for 1 minute followed by 64ºC for 1 minute, 72ºC for 1 minute. The final extension included a cycle of 72ºC for 5 minutes. The multiplex PCR analysis of GSTT1 and GSTM1 genes are presented in figure (I) where the wild genotype of GSTM1 is presented as a band of 215 bp and the wild genotype of GSTT1 is presented as a band of 480 bp. No bands were detected with GSTM1, GSTM1 null genotypes

(homozygous absence or deletion). Albumin used as internal positive control (show 350 bp band), in order to discriminate the null genotype from aborted PCR reactions.

PON1 genotype analysis:

The genotypes of PON1 gene was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the method described by **Humbert et al. (1993)** and **Zama et al. (1997).** The primer used for amplification was forward: 5`-TAT TGT TGC TGT GGG ACC TGA G-3` and reverse: 5`-CAC GCT AAA CCC AAA TAC ATC TC-3`. The amplification was achieved using a thermal cycler PTC-100 machine (MJ Research, Inc., Watertown, MA). Initial denaturation at 95ºC for 5 minutes was first carried out followed by 35 cycles composed of 95ºC for 1 minute, 58ºC (PON192 fragment) for 1 minute and 72ºC for 1 minute followed lastly by a final step of extension of 72ºC for 7 minutes. For Q192R genotyping, ten microliters of PCR product was digested with 3 units of the restriction enzyme NlaIII and 8 units of the restriction enzyme AlwI (New England Biolabs) at 37ºC for 5 hours. The PCR genotyping was done in a total amount of 25 µl composed of 100 ng of template DNA, 12.5 µl of 2X i-TaqTM PCR Master Mix (iNtRON Biotechnology) and 25 pmol of each primer (Biosearch Technologies, Novato, CA).

The PON192 Q (glutamine) allele was assigned through the presence of a 99 bp (undigested) fragment while R (argenine) was assigned by the presence of 33 bp (digested) fragment. Control DNA samples with identified genotypes were involved in each run to insure accurate genotype detection.

The PON1 PCR products were split in a 3% agarose electrophoresis system from Maxicell, EC 360 M-E-C Apparatus Corporation, St. Petersburg, FL. UV trans-illumination with 100-bp SiZerTM DNA marker (iNtRON Biotechnology) was used for visualization after staining with ethidium bromide as shown in figure II.

Statistical analysis

Data were collected, tabulated, and managed using Statistical Package for Social Science version 16 (SPSS Inc., Chicago, IL). Chi square was used to estimate the statistical significance of dialkyl phosphate metabolites detection in urine of patients. The odds ratio (OR) and its 95% confidence interval (95% CI) was estimated to assess the strength of the association between genotypes, alleles and disease risk. P value less than 0.05 was considered statistically significant.

RESULTS

As shown in table (1), analysis of the socio-demographic information of the 110 cases of chronic kidney disease of unknown etiology presented to Nephrology Unit of Internal Medicine Department, Zagazig University Hospitals from June 2015 to December 2016 revealed that their age ranges from 20 to 50 years, males presents 54.5% of the total number. 63.6% of cases live in rural areas and 52.7% are occupationally exposed to pesticides.

Organophosphorus metabolites in urine:

As shown in **table (2),** GC-MS analysis of urine samples revealed that 48 (82.7%) of cases have detectable DAPs compared to 16 (27.5%) of controls. Whereas population who showed positive results for OP metabolites in urine were subjected to DNA extraction and genotyping.

Analysis of GSM1and GSTT1 gene deletions and risk of CKDu:

The frequencies of genotype were adapted to the Hardy–Weinberg equilibrium in patients (P = 0.932) and in controls (P = 0.917). The distribution of GSTM1 genotypes and allele frequencies in CKDu patients and controls were presented in **table (3)**. Out of total 48 patients, the GSTM1 null allele was detected in 35 (72.9%) and the GSTM1 wild alleles was detected in 13 (27.1%). Of the 16 control subjects, the null allele was found in 5 (31.2%), wild GSTM1 present in 11 (68.8%). The difference was statistically significant (p= 0.007) and the Odds ratio was 5.9 with 95% confidence interval 1.7-20.3

The GSTT1 genotype distribution and allele frequencies in CKDu patients and controls were presented in **table (4)**. Out of total 48 CKDu patients, GSTT1 null allele was observed in 29 (60.4%) and the wild alleles was observed in 19 (39.6%). Of the 16 controls, GSTT1 null allele was observed in 4 subjects (25%), GSTT1 present in 12 subjects (75%). The difference was statistically significant (p= 0.03) and the Odds ratio was 4.5 with 95% confidence interval 1.2-16.3

Analysis of PON1 Q192R polymorphism:

The Q192R genotypes distribution was also harmonious with Hardy–Weinberg equilibrium in CKDu patients (P = 0.791) and in controls (P = 0.710). The PON1 Q192R genotype distribution and allele frequencies is shown in table (5) where QQ (homozygous), QR (heterozygous) and RR (homozygous mutated) were observed in 27.1%, 14.5% and 58.4% of CKDu patients respectively versus 68.7%, 12.5% and 18.8% in control subjects respectively. The R mutated allele was detected in 65.6% of CKDu patients and 25% of controls. Regarding the risk of CKDu development, the QQ wild genotype and Q wild allele were taken as references. The study revealed that subjects who are QR heterozygotes showed no risk for CKDu (OR 0.8, 95% CI 0.1 - 4.5, P = 0.6) while RR homozygotes, and R allele showed high risk for CKDu (OR 0.1, 95% CI 0.04-0.6, P = 0.01) (OR 5.7, 95% CI 2.3-14.1, P =0.0001) respectively.

Table (1): Socio-demographic characteristics of cases of chronic kidney disease of unknown etiology

Variables	Number	%
Age	35±15	
Sex		
-Males	60	54.5
-Females	50	45.5
Residence		
-urban	40	36.4
-rural	70	63.6
occupation		
-Exposed to pesticide	58	52.7
-Not exposed to pesticide	52	47.3
Duration of pesticide exposure		
- 0	52	47.3
-less than 10 years	14	12.7
-10 years or more	44	40.0
Total	110	100%

Table (2): statistical comparison of detectable dialkyl phosphate metabolites in urine between cases of CKDu and controls using Chi- square test

	CKDu patients		Controls		P value
	Number	%	Number	%	
Detectable DAPs	48	82.7	16	27.5	0.000
Non -detectable DAPs	10	17.3	42	72.5	
Total	58	100	58	100	

P < 0.05: statistically significant.

Table (3): Statistical comparison of the genotype distribution of the GSTM1 in CKDu patients and controls using Chi-square test

Group / GSTM1	CKDu cases		control		P	OR (95% CI)
	Number	%	Number	%		
Present	13	27.1	5	31.2	0.007	5.9 (1.7-20.3)
null	35	72.9	11	68.8		
Total	48	100	16	100		

P < 0.05: statistically significant CI: confidence interval OR:odds ratio

Table (4): Statistical comparison of the genotype distribution of GSTT1 in CKDu patients and controls using Chi-square test

Group / GSTT1	CKDu cases		control		P	OR (95% CI)
	Number	%	Number	%		
Present	19	39.6	12	75	0.03	4.5 (1.2-16.3)
null	29	60.4	4	25		
Total	48	100	16	100		

P < 0.05: statistically significant OR = odds ratio CI = confidence interval

Table (5): Statistical comparison of the distribution of PON1 Q192R allele and genotype frequencies in CKDu patients and the controls using Chi-square test

Groups / PON1 Q192R	CKDu cases		control		P	OR (95% CI)
	Number	%	Number	%		
Genotypes					-------	
QQ	13	27.1	11	68.7	0.6	1.0 (reference)
QR	7	14.5	2	12.5		0.8 (0.1-4.5)
RR	28	58.4	3	18.8	0.01	0.1 (0.04-0.6)
Total	48	100	16	100		
Alleles						
Q	33	34.4	24	75.0	---------	1.0 (reference)
R	63	65.6	8	25.0	0.0001	5.7 (2.3-14.1)
Total	96	100	32	100		

P < 0.05: significant OR: Odds Ratio 95% CI: 95% confidence interval

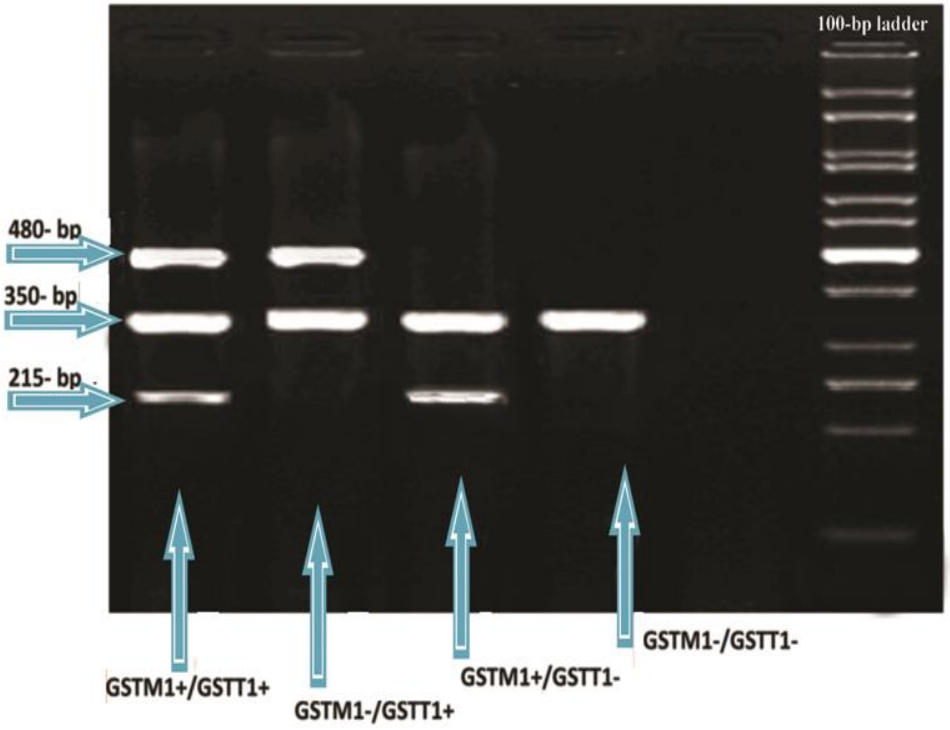

Figure (I): Multiplex PCR patterns for GSTM1 and GSTT1 genes.

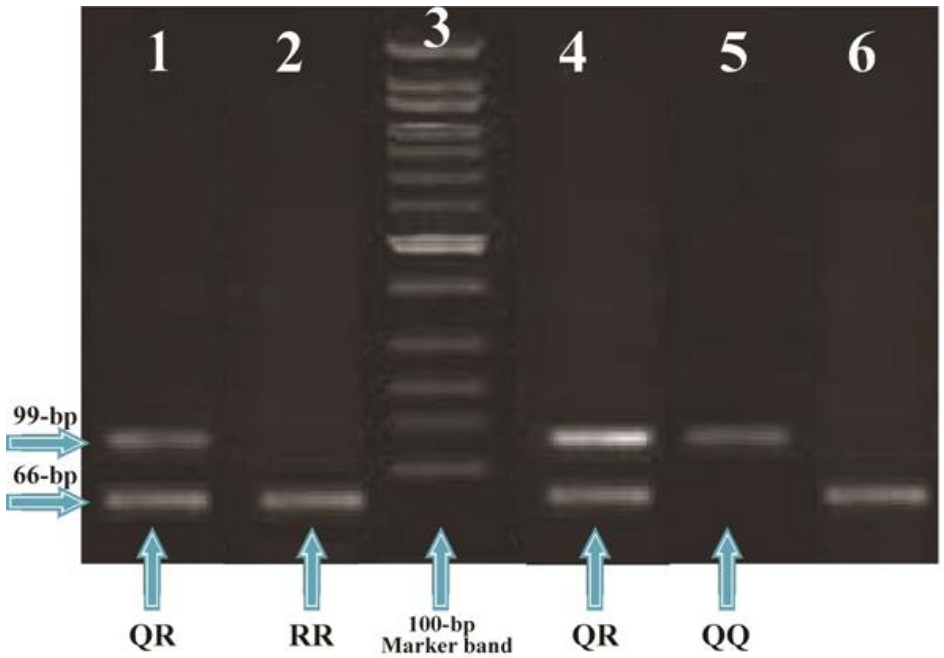

Figure (II): Agarose gel electrophoresis showing the bands pattern obtained by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) for genotyping the PON1 Q192R polymorphism; lane 1, 4 for QR with 99-bp and 66-bp bands; lane 2 for RR with 66-bp; Lane 5 contains a 99-bp represent QQ genotype; and 44-bp (not shown) bands and lane 3, contains a 100-bp marker bands.

DISCUSSION

Numerous current studies have attracted attention to the appearance of an epidemic of CKD not ascribed to ordinary etiologies such as hypertension, diabetes, obstructive uropathy or glomerulonephritis in Central America, southern Asia and Egypt **(Jayatilake et al., 2013). Jayasinghe (2014)** stated that risk of CKDu increases with rural agricultural work and agrochemical exposure; hence the condition should be retitled chronic agrochemical nephropathy to emphasize the likely etiology. The aim of the present work was to investigate the association of genetic polymorphism of GST (M1, T1) and PON1 Q192R with CKDu in pesticide exposed patients.

In the current work 110 cases met the criteria of CKDu; analysis of the socio-demographic data of those patients demonstrated that they ranged from 20 to 50 years in age which correlates with the mean age of CKDu patients in other areas of the world as India, Sri Lanka and Central America **(Almaquer et al., 2014; Wijetunge et al., 2015).** Prevalence was generally higher in male subjects and those living in rural areas in accordance with **Kamel and El-Minshawy (2010); Weaver et al. (2015).** Among those occupationally exposed to pesticide it was found that a higher proportion of patients were exposed for 10 years or more; coinciding with these results **Valcke et al. (2017)** found in his review some evidence of association between pesticides exposure and CKDu, more clearly in studies with stronger design and better exposure assessment.

In the current work urinary dialkyl phosphate metabolite was used as a

marker of chronic exposure to organophosphate compounds. Although red blood cell choline esterase activity can also be used as a marker of chronic exposure to organophosphates, it has the advantage of difficult interpretation due to inter- and intra-individual variation and the absence of baseline values for individuals **(Wessels et al., 2003).** According to **Meeker et al. (2005)** urinary organophosphate metabolites are often the preferred method for monitoring exposure because sample collection is easy and non-invasive and they are measured easily. In addition, **Cocker et al. (2002)** stated that urinary organophosphate metabolites are more sensitive than choline esterase activity because it can be detected at lower levels of exposure.

Out of fifty eight cases of CKDu included in the study, forty eight showed positive urinary dialkyl phosphate metabolites coinciding with **Jayatilake et al. (2013)** who reported increase in urinary pesticide residues above reference levels in 31.6% of CKDu cases. In addition, some scientists have shown that water in endemic areas of CKDu is heavily contaminated with pesticides and their residues **(Jayasumana et al., 2014).**

Our work declared that there is a significant correlation between GSTM1, GSTT1 null genotypes and development of CKDu in patients chronically exposed to organophosphates. In accordance with these results **Agrawal et al. (2007)** stated that the null / low polymorphism of the detoxifying enzymes GSTT1, GSTM1, and GSTP1 are linked to increasing risk of developing ESRD in North Indian patients. In addition, **Gutiérrez-Amavizca et al. (2013)** reported GSTM1 null genotype in 61% of ESRD patients. Null polymorphism represents the homozygous deletion of the gene **(Peddireddy et al., 2016)**. As GSTM1 and GSTT1 are entangled in handling lipid peroxidation products, reactive oxygen species and various central metabolites of toxic substances, there is hypothetical relation between genetic polymorphism of those enzymes and the developing chronic diseases **(Cilensek et al., 2012). Datta et al. (2010)** detected that GSTM1 and GSTT1 genes deletion was associated with higher oxidative stress and lower GST levels in non-diabetic and diabetic chronic kidney disease. Moreover, according to **Singh et al. (2011a)** and **Tumer et al. (2016)** GSTM1 null genotype was associated with higher incidence of DNA damage in workers occupationally exposed to pesticides compared to controls; they correlated this to the affection of metabolism of these chemicals by the genetic variation of glutathione S-transferases. Also, higher DNA damage was detected in pesticide exposed subjects with GSTT1 gene deletion or concurrent deletion of GSTT1 and GSTM1 **(Abhishek et al. (2010).**

This work detected that subjects chronically exposed to OP and PON1 Q192R heterozygous showed no risk for developing CKDu while RR homozygous showed high risk for CKDu.

PON1 192 RR genotype was also previously found to have an association with other chronic diseases as type 2 diabetes mellitus **(El-Lebedy et al., 2014)**. In addition, **Li et al. (2006)** reported that workers occupationally exposed to lead who are homozygous for the R allele are more susceptible to lead toxicity than are subjects of other genotypes

Human paraoxonase 1 (PON1) is an enzyme linked to lipoprotein and implicated in the detoxification of chemicals as organophosphate pesticide compounds by hydrolyzing the bioactive oxons. Polymorphism of the PON1 gene is responsible for discrepancy in the expression and catalytic activity of PON1 enzyme **(Singh et al., 2011b).** Significantly decreased PON1 activity was found in chronic kidney disease (CKD) and renal transplant patients **(Paragh et al., 2009). Kamal et al. (2011)** reported that among the different paraoxonase genotypes of lead exposed workers, the enzyme activity was reported to be lowest in RR type. In contrast, **Singh et al. (2011b)** reported that the PON1 activity was found to be significantly higher in the R/R genotypes. The cause for these disagreeing findings may be the broad inter-ethnic variability in PON1 polymorphism noticed in these researches **(Rahmani et al., 2002).**

PON1 has gained great attention after the recognition of its antioxidant characters, especially its ability to protect low density lipoprotein from oxidative damage **(El-Lebedy et al., 2014).** According to **Prakash et al. (2010)** enhancement and maintenance PON1 activity may have anti-atherogenic role and may prevent its complication in patients with chronic renal failure.

CONCLUSION

This work suggested the presence of an association between GSTM1 and GSTT1 null genotypes and PON 1 gene polymorphism and developing CKDu in patients occupationally exposed to organophosphates. Additional studies searching for more possible underlying mechanisms clarifying the pathogenesis of CKDu are necessary, which will allow the prediction of individuals at risk.

CONFLICTS OF INTEREST

No conflict of interest.

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التحور الجينى للجلوتاثيون-أس- ترانسفيراز(ميو١،ثيتا ١) و للباروكسينيز ١ وقابلية الإصابة بأمراض الكلى المزمنة (من النوع الغير معلوم السبب)، في المرضي المعرضين للمبيدات تعرضا مزمنا فى مستشفيات جامعة الزقازيق

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الملخص العربى

وتستخدم مركبات المبيدات عادة في جميع أنحاء العالم وفي مصر. وقد أدى التعرض البشري المزمن لمستوى منخفض لهذه المركبات إلى العديد من الآثار الضارة بالصحة. وقد اقترح وجود ارتباط بين الاصابة بمرض الكلي المزمن (من النوع الغير معلوم السبب) والأنماط الجينية الباطلة فى المرضى .الباروكسينيز -١هو إنزيم مرتبط بالبروتين الدهني عالي الكثافة الذى يمنع أكسدة البروتينات الدهنية منخفضة الكثافة ويشارك في إزالة المبيدات الفوسفاتية العضوية. الجلوتاثيون - أس-ترانسفيراز- ميو١ والجلوتاثيون -أس-ترانسفيراز- ثيتا ١ هم أعضاء في جماعة المرحلة الثانية من إنزيمات تأييض المواد الدخيلة بما في ذلك المبيدات الفوسفاتية العضوية. جينات الجلوتاثيون - أس- ترانسفيراز- ميو١ والجلوتاثيون -أس-ترانسفيراز- ثيتا ١ لديها (الانماط الباطلة) التي بها الجين مختفيا بالكاملز

وكان الهدف من هذه الدراسة الحالية هو التحقق من العلاقة بين الأنماط الجينية الباطلة من الجلوتاثيون - أس- ترانسفيراز- ميو١ والجلوتاثيون -أس-ترانسفيراز- ثيتا ١و الأشكال المتعددة للباروكسينيز - ١ مع مرض الكلي المزمن (من النوع الغير معلوم السبب)، في المرضى المعرضين للمبيدات عرضت على وحدة أمراض الكلى، قسم الطب الباطني، ومستشفيات جامعة الزقازيق، محافظة الشرقية .

واستخدمت عينات البول للكشف عن الألكيل الفوسفات لدى المرضي المدرجين في الدراسة باستخدام الجهاز اللوني للغاز مطياف الكتلة ، ثم إستخدام التفاعل التسلسلي عديد البلمرة للكشف عن وجود أو عدم وجود جينات الجلوتاثيون - أس- ترانسفيراز- ميو١ والجلوتاثيون - أس- ترانسفيراز- ثيتا ١ (حذف الجينات) و التنميط الجينى لأشكال الباروكسينيز – ١ المتعددة بإستخدام طريقة التفاعل التسلسلي عديد البلمرة مع استخدام إنزيم محدد القطع في الحمض النووي الجيني المعزول فى عينات دم من ٤٨ مريض كلي مزمن (من النوع الغير معلوم السبب) لتعرضهم المزمن للمبيدات الحشرية ومطابقتهم للمجموعة الضابطة.

. وأظهرت نتائج هذه الدراسة أن هناك تأثير للتعرض المزمن للمبيدات الفوسفاتية العضوية علي زيادة احتمالية الاصابة بمرض الكلي المزمن (من النوع الغير معلوم السبب) مقارنة بالمجموعة الضابطة