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Pesticide, Gene Polymorphisms and Bladder Cancer among Egyptian Agricultural Workers

Sania Amr, MD, MS, **Rebecca Dawson, PhD, MPH**, **Doa'a A. Saleh, MD, PhD**, **Laurence S. Magder, PhD**, **Diane Marie St. George, PhD**, **Mai El-Daly, PhD**, **Katherine Squibb, PhD**, **Nabiel N. Mikhail, PhD**, **Mohamed Abdel-Hamid, PhD**, **Hussein Khaled, MD, PhD**, and **Christopher A. Loffredo, PhD**

Department of Epidemiology and Public Health (S Amr, R Dawson, L Magder, DM St. George) and Department of Medicine (K Squibb), University of Maryland School of Medicine, Baltimore, MD; Lombardi Cancer Center, Georgetown University Medical Center (CA Loffredo), Washington, DC; Department of Chemotherapy, National Cancer Institute (H Khaled) and Department of Community Medicine, Cairo University (DA Saleh), Cairo Egypt; National Liver Institute, University of Menoufia (M El-Daly), Egypt; Department of Biostatistics and Cancer Epidemiology, South Egypt Cancer Institute, Assiut University (NN Mikhail), Egypt; and Department of Microbiology and Immunology, Minia University (M Abdel-Hamid), Egypt

Abstract

We examined the associations between pesticide exposure, genetic polymorphisms for NAD(P)H:quinone oxidoreductase I (*NQO1*) and superoxide dismutase 2 (*SOD2*), and urinary bladder cancer risk among male agricultural workers in Egypt.

We used logistic regression to analyze data from a multi-center case-control study and estimate adjusted odds ratio (OR) and 95% CI (confidence interval)

Exposure to pesticides was associated with increased bladder cancer risk (1.68 (1.23–2.29)) in a dose-dependent manner. The association was slightly stronger for urothelial (1.79 (1.25–2.56) than for squamous cell carcinoma (1.55 (1.03–2.31)), and among participants with combined genotypes for low *NQO1* and high *SOD2* (2.14 (1.19–3.85) activities as compared to those with high *NQO1* and low *SOD2* genotypes (1.53 (0.73–3.25)).

In conclusion, among male agricultural workers in Egypt, pesticide exposure is associated with bladder cancer risk and possibly modulated by genetic polymorphism.

Keywords

Pesticides; Bladder cancer; Gene polymorphism; Epidemiology; Egypt; Agricultural workers

Address correspondence to: Sania Amr, MD, MS, Department of Epidemiology and Public Health, University of Maryland School of Medicine, 660 West Redwood Street, Suite 133C Howard Hall, Baltimore, MD 21201, samr@epi.umaryland.edu.

Introduction

Tobacco smoking, history of infection with *Shistosoma hematobium* (SH), and occupational chemical exposures have been well-established as risk factors for urinary bladder cancer;[1–6] namely the two predominant types (95%), urothelial cell carcinoma (UC) and squamous cell carcinoma (SCC). The association of farming as an occupation with bladder cancer risk was examined by several investigators, but the results are inconsistent; some studies found an increased risk,[7–11] while others reported decreased risk.[12,13] Agricultural workers are exposed to chemicals including aromatic amines and polycyclic aromatic hydrocarbon, which were consistently found to increase the risk for bladder cancer,[3,4,6] and pesticides; the latter encompasses an array of compounds with different metabolic pathways and actions.[14] Indeed, some pesticides are considered probable or possible carcinogens,[15] and few were found to be associated with increased risk of bladder cancer,[16–19] but not others.[20–21]

Considering that metabolism of some pesticides can lead to an increase in the levels of reactive oxygen species (ROS),[14,22,23] Investigators have examined the roles of polymorphism in genes encoding for enzymes that play key roles in protecting the cells from oxidative injury, one of the potential mechanism underlying carcinogenesis. Among the several genetic variants that were studied in different malignancies[24–26] are those that code for two enzymes, NAD(P)H:quinone oxidoreductase I (*NQO1*)[27,28] and superoxide dismutase 2 (*SOD2*).[29] A transition from C→T at nucleotide 609 of *NQO1* (rs1800566) and in exon 2 of *SOD2* (rs4880) results in proline to serine and alanine to valine substitution, respectively; changes in the enzyme structures lead to decrease in their activities,[30,31] and thus increase in oxidative stress and cell susceptibility. So far, the studies aimed at investigating the associations of genetic susceptibility and environmental exposures with bladder cancer risk have focused mainly on gene interaction with tobacco smoking; very few studies addressed *NQO1* and *SOD2* genotypes and pesticide exposures. In addition these investigations have been conducted among populations where UC is the predominant (~90%) type of primary bladder malignancy, and they have yielded conflicting results.[32–41]

Using data from our multicenter case-control study of bladder cancer in Egypt where more than 30% of the cases are of the SCC type,[42] we recently found that Egyptian male agricultural workers have increased odds of developing bladder cancer as compared to other workers[7], after adjustment for smoking and SH infection history. We also found that genetic polymorphisms in *NQO1* and *SOD2* modulate bladder cancer risk associated with smoking and history of SH infection.[43] In the present study, we used the same population to examine: 1) the associations between bladder cancer risk and pesticide exposure among male agricultural workers; and 2) to investigate potential interactions between such exposures and genetic polymorphism of *NQO1* and *SOD2*.

Methods

We analyzed data from a multicenter case control study conducted in Egypt between 2006 and 2011. The study was approved by the Institutional Review Boards of the three recruitment sites described below, Egypt's Ministry of Health, the University of Maryland Baltimore, and Georgetown University.

Study Population

Recruitment for the study population were previously described in detail.[7,42] Briefly, cases were recruited from three referral cancer centers that serve a large area of Egypt: the National Cancer Institute of Cairo University, the Minia Oncology Center, and the South Egypt Cancer Center in Assiut. The diagnosis of primary urinary bladder cancer was ascertained by one of the study's two pathologists. We only included primary cases classified as UC or SCC in the present analyses. Healthy controls were randomly selected to frequency-match the cumulative groups of cases by sex, five-year age groups, governorate (province) of current residence, and urban/rural place of residence as self-reported.[42]

After consenting, cases and controls were questioned face-to-face by trained interviewers, who collected data on socio-demographic characteristics, occupational and environmental exposure histories including pesticide exposure, frequency, and duration, tobacco smoking (including cigarettes and waterpipes), and medical histories including history of SH infection. Blood specimens were drawn and processed for DNA extraction.

Of the 3,427 presumed bladder cancer cases who were eligible, 84% agreed to participate in the study; and of the 2,792 eligible controls approached, 97% agreed. Among the cases confirmed by the pathologist as UC or SCC, 1,525 were males matched to 2069 controls. For the present study, we included only those males who reported farming as an occupation (953 cases and 881 controls). Because of lack of funding, genotyping was carried out on 810 controls and 1356 cases only. Among those genotyped, 822 were agricultural workers, the majority of whom (N=777, comprising 419 cases and 358 controls) was from rural south Egypt.

Genotyping

DNA was extracted from buffy coats using the QIAamp DNA Mini Kits (Qiagen, Valencia, CA, USA). Genotyping for *NQO1* Pro187Ser polymorphism (rs1800566) and *SOD2* Ala-9Val polymorphism (rs4880) was performed using TaqMan allelic discrimination assays (Applied Biosystems), with a successful genotyping rate of 99% or higher, and genotype concordance among 10% of blind quality control duplicates of > 99.

Variables

The independent variables of interest were occupational exposure to pesticides (yes/no), frequency of pesticide exposure (categorical), and duration of exposure in years (continuous and categorical). Based on the distribution of the exposure duration, we divided it into four categories, never, ≤ 20 years, > 20 but ≤ 40 years, and > 40years. The outcome variable was primary bladder cancer or its subtypes (SCC or UC) case versus control. Covariates that were examined and included in the analyses were education, tobacco smoking history (either cigarettes or waterpipes alone, or combined), environmental tobacco smoke (ETS) exposure, and SH infection history. Regarding area of residence, Egypt has 27 governorates that are relatively homogenous within the broad regions (North and South). Because we had few cases from some of the governorates, we grouped the area of residence in two categories, North and South, for adjustment in the analyses, in addition to adjusting for urban versus rural. Furthermore, we generated four categories of age interval (≤ 45; 45 < ≤55; 55< ≤ 65;

and > 65 years) for descriptive analyses, and regrouped them into three categories for stratified analyses. For the *NQO1* genotype, the main polymorphisms of interest were CC, TC, and TT, encoding for high, intermediate and low activity, respectively. Because the number of specimen with TT for *NQO1* was very small, TT and TC were combined. For the *SOD2* genotype, the polymorphisms of interest were CC, TC, and TT, encoding respectively for high, intermediate, and low activity. We also generated two variables, one for combined *NQO1* (TT or TC) with *SOD2* (CC or TC), and another for combined *NQO1* (CC) with *SOD2* (TT).

Statistical Analyses

We used descriptive analyses to compare socio-demographic characteristics, SH infection history, and exposure to pesticides and its frequency and duration between cases and controls; Chi square and Student's t test were used to compare categorical and continuous variables, respectively.

To assess the relationship between pesticides exposure and bladder cancer, we conducted separate analyses for each of the exposure parameter, i.e., exposure (yes/no), frequency, and duration. We used logistic regression to estimate odds ratios (OR) and 95% confidence interval (CI) for associations between independent variables and covariates, and bladder cancer risk. We tested each covariate for potential confounding or effect modification of the association between independent variable and outcome. In the final models we adjusted for significant covariates (education, tobacco smoke, SH infection history, and ETS) and the matching variables, age and area of residence (north versus south and urban versus rural). We used polytomous regression to simultaneously examine the associations between pesticides exposure and UC and SCC risk. In addition we stratified the sample by age interval (≤ 55; 55 < ≤ 65; and > 65) and performed analyses of pesticides exposure and its duration and risk of bladder cancer for each of the strata.

The analyses for gene polymorphisms and their interactions with pesticides were limited to those male agricultural workers from rural south Egypt, and thus were not adjusted for area of residence. The dataset(s) that were used in final analyses did not include missing variables, or in the case of the genotypes those with undetermined results, and therefore the sample sizes indicated in the tables might slightly differ from the size of the original sample. All statistical analyses were performed using SAS version 9.2 software.

Results

In our study population, we had 953 primary urinary bladder carcinoma confirmed as UC or SCC cases, and 881 controls, all of whom reported being agricultural workers. Their characteristics are shown in table 1. The majority was from rural south Egypt. Less than 20% of the cases and the controls never smoked.

Among agricultural workers for whom DNA specimen were genotyped for *NQO1* and *SOD2*, there were 419 cases and 358 controls who resided in rural south Egypt. The characteristics of this subset were comparable to those of the larger sample of agricultural

workers (Table 1). The distributions of the CC, TC, and TT genotypes for *NQO1* and *SOD2* were similar among cases and controls *(p= 0.98)* (Table 1).

As shown in table 2, more cases than controls were exposed to pesticides (56.7% versus 51.3% in the large sample) and for longer duration (35.3 versus 29.1 years). Exposure to pesticides was associated with increased odds of having bladder cancer among male agricultural workers, even after adjustment for tobacco smoke, SH history, and other covariates, albeit not statistically significant (OR (95% CI), 1.16 (0.95–1.41)); however, there were significant trends with increased frequency ($p = 0.04$) and more so with duration of exposure ($p < 0.0001$). We found that the longer the duration of pesticide exposure, the higher were the odds of developing bladder cancer; the OR (95% CI) for over 40 years of exposure was 2.18 (1.62–2.95), higher than the OR for an exposure duration between 20 and 40 years (1.14 (0.87–1.50)) (Table 2). We also found that adjusted ORs were 1.26 (1.01–1.58) and 1.01 (0.77–1.31), for UC and SCC, respectively, in the large sample of agricultural workers.

Among participants who were genotyped and lived in rural south Egypt, the adjusted OR for having bladder cancer was greater (1.68 (1.23–2.29)) than the OR among all agricultural workers (1.16 (0.95–1.41)) (Table 2), but like among the latter it significantly increased with the exposure frequency and duration (p for trend < 0.0001).

Whether in the bivariate or multivariable analyses, *SOD2* and *NQO1* genotypes were not statistically significantly associated with bladder cancer risk. We performed multivariable regression analyses where we included pesticide exposure, genotype, their interaction term, and covariates to estimate the association between pesticide exposure and cancer risk separately among those with high or low activity enzyme genotypes, and the significance of the interaction. Table 3 shows the adjusted estimates of associations between pesticide exposure and bladder cancer in different strata of genotypes.

Exposure to pesticides was associated with increased odds of having bladder cancer regardless of the genotype, albeit such increases varied in magnitude and statistical significance. For *NQO1*, the odds ratios were slightly higher among those with low (TT) or intermediate (TC) activity (1.94 (1.20–3.14)) as compared to those with high activity (CC) (1.49 (0.99–2.22)), while for the *SOD2* strata, the odds ratios were not different between the most active form (CC) (1.74 (1.02–2.99)) of the enzyme as compared to the least active (TT) (1.68 (1.02–2.79)). The interaction term for pesticide exposure with either genotype was not statistically significant ($p = 0.40$ and 0.93, for *NQO1*and *SOD2*, respectively) (Table 3).

Exposure to pesticides was associated with increased odds of having either type of bladder cancer, and the association was somewhat stronger for UC than for SCC (OR (95% CI) 1.79 (1.25–2.56)) versus (1.55 (1.03–2.31)); this pattern was consistently seen across the different genotypes, although the differences across the two cancer subtypes were not statistically significant (Table 3).

We also examined the effects of pesticide exposure on bladder cancer risk among those with combined genotypes: low and intermediate activity for *NQO1* (TT orTC) with high and intermediate activity for *SOD2* (CC or TC) versus high activity for *NQO1*(CC) combined

with low activity for *SOD2* (TT). Table 3 shows that among participants with the former combination the OR for having either type of bladder cancer was greater (2.14 (1.19–3.85)) than the OR among those with the former (1.53 (0.73–3.25)), and it was slightly higher than the ORs observed separately with either the low and intermediate activities *NQO1* (TT and TC) (1.94 (1.20–3.14)) or the high activity *SOD2* (CC) (1.74 (1.02–2.99)) (Table 3).

To reduce residual confounding by age, despite adjustment for it, we stratified the sample by age group and assessed the duration of pesticide exposure among different strata. The adjusted OR (95% CI) for being exposed for ≥ 40 years were 2.94 (1.12–7.75), 2.22 (1.36–3.62), and 2.01 (1.30–3.11) for those who were ≤ 55, 55 < ≤ 65, and > 65 year-olds, respectively.

Discussion

We examined the associations between pesticide exposure and bladder cancer risk among male agricultural workers in Egypt. We found that exposure to pesticides increased the odds of having bladder cancer, in a dose-dependent manner (the longer the exposure the greater were the odds), even after adjustment for other known risk factors such as tobacco smoke and SH infection history. The association between pesticide exposure and bladder cancer risk was somewhat stronger for the UC than the SCC type, but the difference between the two subtypes was not statistically significant. Furthermore, the increase in the odds for this malignancy associated with pesticide exposure was greater for those with low or intermediate activity than high activity genotype for the enzyme *NQO1*.

Our finding that exposure to pesticides is positively associated with increased bladder cancer risk are consistent with some of the previously reported results.[16–19] The fact that increased frequency and duration of exposure were positively associated with bladder cancer is novel and provides further support for such an association. Indeed, some pesticides are known to be associated with malignancies,[4,15,44–46] and a few were found to be associated with bladder cancer;[16–19] albeit the association was not statistically always significant.[16,18]

We observed that pesticide exposure was statistically significantly associated with both types of bladder cancer, SCC and UC. Although the association estimates were not statistically significantly different between the two subtypes, the ORs for UC risk was consistently higher than that of SCC across all genotypes analyzed, and the risk for the former was more frequently statistically significant (Table 3). The inconsistencies in significance could potentially be attributed to small sample size; SCC cases being fewer than UC.

We also found the OR of the association between pesticides and bladder malignancy to be higher among participants with genotypes coding for low and intermediate enzyme activity (TT and TC) as compared to those coding for high activity (CC) *NQO1* (Table 3). Previous studies have reported that this genetic polymorphism in *NQO1* leads to different susceptibility to develop bladder cancer risk,[28,32,33,30,35,41] by modifying the risk of cigarette smoking.[35,37,39] Our study is the first to address *NQO1* genotype interaction with pesticide exposures.

Several investigators, including ourselves, examined *SOD2* genotype and bladder cancer risk relationships,[34,36,38,43] but the focus was on gene interaction with smoking. The present study is the first to address *SOD2* polymorphism interaction with pesticide exposure and bladder cancer risk. We found with *SOD2* genotypes somewhat the reverse of our finding with *NQO1*, especially for UC risk; that is, genotypes for high and intermediate enzyme activity (CC or TC) were associated with greater odds of having bladder cancer than the genotype with low activity among pesticide-exposed agricultural workers (Table 3). The increased risk of cancer with the active form of SOD2 genotype, although unexpected, was previously reported in a study of prostate cancer, smoking and vitamin E intake.[47] A possible explanation is that the active form of *SOD2* can lead to an increase in intermediate metabolites of ROS with potentially stronger carcinogenic effects than the original substrate; active forms of *SOD2* lead to production of hydrogen peroxide (H_2O_2) which, in turn can damage DNA and participate in carcinogenesis.[48,49] Indeed, such a mechanism was demonstrated for the enzymes encoded by gene variants of the glutathione S-transferase,[50,51] and for catechol-O-methyltransferase.[52] Our finding that agricultural workers with combined low activity *NQO1* and high activity *SOD2* genotypes had slightly higher risk than those with either one alone can possibly be due to an accumulation of ROS as results of its increased production by an active *SOD2* and decreased removal by a less active *NQO1*.

The associations between bladder cancer risk and pesticides was stronger in the sample restricted to south rural Egypt (1.68 (1.23–2.29)) as compared to the main sample (1.16 (0.95–1.41)); and that was mostly driven by a proportion of pesticides-exposed cases from this area (63%) that is larger than that in the sample from all over Egypt (56%).

One of our study limitations is that we did not inquire about the specific types of pesticides that were used in agriculture. In a previous study, Ezzat et al.[45] reported a long list of pesticides to which agricultural workers are exposed in Egypt; most of the major chemical categories are included, and one can assume that agricultural workers are exposed to a mixture of chemicals. Our study is one of the largest case-control studies reported on bladder cancer malignancy and pesticides, and where we were able to address its heterogeneity by examining UC and SCC separately.

The genetic analyses and gene-environment interaction analysis were performed on a smaller sample that was restricted to the rural south area, and thus the results might not be representative of the general study population. The distributions of *NQO1* and *SOD2* alleles among cases and controls of our restricted sample were similar to those we observed in a larger sample of genotyped cases and controls we investigated for interaction between these genes and smoking and SH infection.[43]

In conclusion, we found that male agricultural workers in Egypt may be at higher risk than other males for developing bladder cancer, a risk that was associated with pesticide exposures and to which contribution by susceptible genetic backgrounds is possible. Future research is needed to address the pathogenetic mechanisms underlying the pathways of carcinogenesis from exposure to specific pesticides.

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Table 1

Characteristics of Bladder Cancer Cases and Controls Among Male Agricultural Workers in Egypt

Characteristic/Exposure	All Agricultural Workers		Genotyped Agricultural Workers*	
	Controls N=881 N (%)	Cases N=953 N (%)	Controls N=358 N (%)	Cases N= 419 N (%)
Mean age ± SD	60.2±11.0	60.7±10.3	58.5±11.7	59.6±10.4
Age group				
≤ 45	91 (10.3%)	71 (7.5%)	56 (15.6%)	38 (9.1%)
45 < ≤ 55	227 (25.8%)	223 (23.4%)	98 (27.4%)	109 (26.0%)
55 < ≤ 65	278 (31.6%)	329 (34.5%)	103 (28.8%	143 (34.1%)
> 65	285 (32.3%)	330 (34.6%)	101 (28.2%)	129 (30.8%)
Residence				
North	41 (4.6)	54 (5.7)	-	-
South	840 (95.3)	899 (94.3)	358 (100%)	419 (100%)
Urban	9 (1.0)	44 (4.6)	-	-
Rural	872 (99.0)	909 (95.4)	358 (100%)	419 (100%)
Education				
None	632 (71.7)	779 (81.8)	271 (75.7)	333 (79.7%)
Some & higher	249 (28.3)	173 (18.2)	87 (24.3%)	85 (20.3%)
Missing		1		1
Tobacco smoke				
Never	161 (18.3)	158 (16.6)	63 (17.6%)	60 (14.3%)
Waterpipe only	115 (13.0)	117 (12.3)	40 (11.2%)	52 (12.4%)
Cigarette only	541 (61.4)	547 (57.4)	227 (63.4%)	243 (58.0%)
Both	64 (7.3)	131 (13.7)	28 (7.8%)	64 (15.3%)
ETS∝				
No	53 (32.9%)	34 (21.5%)	25 (39.7%)	14 (23.3%)
Yes	108 (67.1%)	124 (78.5%)	38 (60.3%)	46 (73.7%)
SH Infection history$				
No	387 (43.9)	361 (37.9)	184 (51.4%)	140 (33.4%)
Yes	436 (49.5)	540 (56.6)	157 (43.8%)	256 (61.1%)
Do not know	58 (6.6)	52 (5.5)	17 (4.8%)	23 (5.5%)
NQO1¶ genotype&				
TT@			19 (5.3%)	22 (5.3%)
TC			127 (35.8%)	146 (35.2%)
CC			209 (58.9%)	247 (59.5%)
Undetermined			3	4

Characteristic/Exposure	All Agricultural Workers		Genotyped Agricultural Workers*	
	Controls N=881 N (%)	Cases N=953 N (%)	Controls N=358 N (%)	Cases N= 419 N (%)
SOD2¶ genotype&				
TT			109 (30.6%)	127 (30.7%)
TC			160 (45.0%)	188 (45.4%)
CC			87 (24.4%)	99 (23.9%)
Undetermined			2	5

*Sample was from rural south Egypt;

∝Environmental tobacco smoke;

$*Shistosoma hematobium* (SH) infection;

¶NQO1 (NAD(P)H:Quinone Oxidoreductase); SOD2 (Superoxide Dismutase);

@TT, TC, and CC, represent the variants of the genes that code respectively for the least active, intermediate, and more active forms of either enzyme;

&$p= 0.98$ for comparing cases with controls using chi square.

Table 2

Associations between Pesticide Exposure, its Frequency and Duration, and Bladder Cancer Risk among Male Agricultural Workers in Egypt

	All Male Agricultural Workers			Genotyped Agricultural Workers from Rural South Egypt		
Pesticide Exposure	**Controls N=881 N (%)**	**Cases N=953 N (%)**	**Adjusted* OR and (95% CI)**	**Controls N=358 N (%)**	**Cases N=419 N (%)**	**Adjusted** OR (95% CI)**
Exposure						
No	429 (48.7)	413 (43.3)	Ref	190 (53.1%)	155 (37.0%)	Ref
Yes	452 (51.3)	540 (56.7)	1.16 (0.95–1.41)	168 (46.9%)	264 (63.0%)	1.68 (1.23–2.29)
Frequency						
None	429 (48.7)	413 (43.3)	Ref	190 (53.1%)	155 (37.0%)	Ref
< once per month	326 (37.0)	395 (41.4)	1.16 (0.94–1.44)	138 (38.5%)	190 (45.4%)	1.44 (1.04–2.00)
≥ once per month	103 (11.7)	138 (14.5)	1.33 (0.98–1.81)	25 (7.0%)	71 (16.9%)	3.24 (1.90–5.53)
Missing	23 (2.6)	7(0.8)		5 (1.4%)	3 (0.7%)	
P for trend			*0.04*	*P for trend*		< 0.0001
Mean Duration ± SD	29.1±15.5	35.3±16.4	*p < 0.0001*	25.6±16.1	32.4±16.6	<0.0001
Duration						
None	429 (48.7)	413 (43.3)	Ref	190 (53.1%)	155 (37.0%)	Ref
≤ 20 years	144 (16.3)	109 (11.4)	0.77 (0.57–1.04)	69 (19.3%)	69 (16.5%)	1.10 (0.72–1.68)
>20 but ≤40 years	156 (17.6)	186 (19.5)	1.14 (0.87–1.50)	43 (12.0%)	90 (21.5%)	2.39 (1.51–3.80)
> 40 years	93 (10.4)	213 (22.4)	2.18 (1.62–2.95)	29 (8.1%)	84 (20.0%)	3.03 (1.82–5.04)
Missing	63 (7.0)	32 (3.4)		27 (7.5%)	21 (5%)	
P for trend			*< 0.0001*	*P for trend*		< 0.0001

*Odds ratios (95% confidence interval) adjusted for education, tobacco smoke, SH infection history, environmental tobacco smoke, age (continuous), and area of residence;

**adjusted for all the above except for area of residence

Table 3

Associations between Pesticides Exposure and Bladder Cancer Risk among Male Agricultural Workers from Rural South Egypt and with Different Genotypes for NAD(P)H: Quinone Oxidoreductase 1 (*NQO1*) and Superoxide Dismutase 2 (*SOD2*)

	All Cases				SCC[&] N = 164	UC[&] N = 255
Genotype	**Exposure**	**Control**	**Case**	**Adjusted Odds Ratio (95% Confidence Interval)[*]**		
All genotypes	No Yes	190 168	155 264	Ref 1.68 (1.23–2.29)	Ref 1.55 (1.03–2.31)	Ref 1.79 (1.25–2.56)
Multivariable Regression Models with Interaction						
NQO1 (TT or TC)[@]	No Yes	83 63	65 103	Ref 1.94 (1.20–3.14)	Ref 1.73 (0.91–3.17)	Ref 2.10 (1.21–3.64)
NQO1 (CC)	No Yes	105 104	88 159	Ref 1.49 (0.99–2.22)	Ref 1.40 (0.83–2.36)	Ref 1.57 (0.98–2.50)
Interaction *p-value*[$]				*0.40*	*0.63*	*0.42*
SOD2 (TT)	No Yes	50 59	39 88	Ref 1.68 (1.02–2.79)	Ref 1.75 (0.90–3.39)	Ref 1.64 (0.91–2.94)
SOD2 (TC)	No Yes	93 67	78 110	Ref 1.71 (1.25–2.34)	Ref 1.58 (1.05–2.37)	Ref 1.82 (1.27–2.62)
SOD2 (CC)	No yes	46 41	35 64	Ref 1.74 (1.02–2.99)	Ref 1.43 (0.71–2.86)	2.03 (1.08–3.79)
Interaction *p-value*				*0.93*	*0.71*	*0.66*
NQO1 (TT or TC) *and SOD2* (CC or TC)	No Yes	60 38	47 71	Ref 2.14 (1.19–3.85)	Ref 1.71 (0.80–3.65)	Ref 2.46 (1.26–4.80)
NQO1 (CC) *and SOD2* (TT)	No Yes	27 34	22 56	Ref 1.53 (0.73–3.25)	Ref 1.28 (0.50–3.29)	Ref 1.75 (0.72–4.27)
Interaction *p-value*				*0.48*	*0.63*	*0.55*

[&]Squamous cell carcinoma (SCC) and urothelial cell carcinoma (UC) the risks of which were estimated using polytomous regression;

[*]Adjusted for education, tobacco smoke, SH infection history, environmental tobacco smoke, and age (continuous);

[@]TT, TC, and CC, represent the variants of the genes that code respectively for the least active, intermediate, and more active forms of either *NQO1* or *SOD2;*

[$]*P-value* for difference between genotypes with respect to effect of pesticide on bladder cancer risk.