Published in final edited form as:
Urol Oncol. 2014 January ; 32(1): . doi:10.1016/j.urolonc.2013.06.016.

Genetic polymorphisms in *NQO1* and *SOD2*: Interactions with smoking, schistosoma infection, and bladder cancer risk in Egypt

David Goerlitz, M.S.[a], **Sania Amr, M.D.**[b], **Chiranjeev Dash, Ph.D.**[a], **Doa'a A. Saleh, M.D.**[c], **Mai El Daly, Ph.D.**[d,g], **Mohamed Abdel-Hamid, Ph.D.**[d,e], **Sherif El Kafrawy, Ph.D.**[d,g,j], **Tamer Hifnawy, M.D.**[f], **Sameera Ezzat, M.D.**[g], **Mohamed A. Abdel-Aziz, M.D.**[h], **Hussein Khaled, M.D.**[i], **Yun-Ling Zheng, M.D.**[a], **Nabiel Mikhail, Ph.D.**[d,h], and **Christopher A. Loffredo, Ph.D.**[a,*]

[a]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

[b]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD

[c]Department of Public Health, Cairo University, Cairo, Egypt

[d]Department of Microbiology, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt

[e]Department of Microbiology, Minia University, Minia, Egypt

[f]Public Health Department, Beni Suif University, Beni Suif, Egypt

[g]National Liver Institute, Menoufiya University, Shibin El Kom, Egypt

[h]Department of Urology, Assiut University, Assiut, Egypt

[i]Department of Medical Oncology, National Cancer Institute, Cairo, Egypt

[j]King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background—Bladder cancer is the most prevalent form of cancer in men among Egyptians, for whom tobacco smoke exposure and *Schistosoma haematobium* (SH) infection are the major risk factors. We hypothesized that functional polymorphisms in NAD(P)H:quinone oxidoreductase 1 (*NQO1*) and superoxide dismutase 2 (*SOD2*), modulators of the effects of reactive oxidative species, can influence an individual's susceptibility to these carcinogenic exposures and hence the risk of bladder cancer.

Methods—We assessed the effects of potential interactions between functional polymorphisms in the *NQO1* and *SOD2* genes and exposure to smoking and SH infection on bladder cancer risk among 902 cases and 804 population-based controls in Egypt. We used unconditional logistic regression to estimate the odds ratios (OR) and confidence intervals (CI) 95%.

Results—Water pipe and cigarette smoking were more strongly associated with cancer risk among individuals with the TT genotype for *SOD2* (OR [CI 95%] = 4.41 [1.86–10.42]) as compared with those with the CC genotype (OR [CI 95%] = 2.26 [0.97–6.74]). Conversely, the risk associated with SH infection was higher among the latter (OR [CI 95%] = 3.59 [2.21–5.84])

*Corresponding author. Tel.: +202-687-3758; fax: +202-784-3034. cal9@georgetown.edu (C.A. Loffredo).

than among the former (OR [CI 95%] = 1.86 [1.33–2.60]). Polymorphisms in *NQO1* genotype showed a similar pattern, but to a much lesser extent. The highest odds for having bladder cancer following SH infection were observed among individuals with the CC genotypes for both *NQO1* and *SOD2* (OR [CI 95%] = 4.41 [2.32–8.38]).

Conclusion—Our findings suggest that genetic polymorphisms in *NQO1* and *SOD2* play important roles in the etiology of bladder cancer by modulating the effects of known contributing factors such as smoking and SH infection.

Keywords

NQO1; *SOD2*; Bladder cancer; Epidemiology; Smoking; Schistosomiasis

1. Introduction

Bladder cancer is the seventh most common malignancy in men worldwide, with 297,300 new cases estimated in 2008 [1]. Rates of bladder cancer are highest in Europe, North America, and Northern Africa, where Egyptian men have the highest incidence (37.1 per 100,000 person-years) and mortality rates (16.3 per 100,000 person-years) [1,2]. Urothelial cell (UC) and squamous cell carcinomas (SCC) are the predominant histological types of bladder cancer. Although it is well established that cigarette smoking and *Schistosoma haematobium* (SH) infection are the main contributing factors to bladder cancer in Egypt [3–6], the mechanisms of carcinogenesis in the bladder at the cellular level are largely unknown.

Cellular responses to oxidative stress and inflammation are hypothesized as the common pathways underlying malignant transformation following exposures to several types of toxicants [7,8]. Furthermore, it has been postulated that polymorphisms in genes encoding for enzymes that mediate the toxicity of reactive oxygen species (ROS) and other carcinogens can modify the epidemiological associations between tobacco smoking and bladder cancer risk [9,10]. One example is the *NQO1* gene that codes for the enzyme NAD(P)H:quinone oxidoreductase 1, which plays an important role in protecting cells from oxidative damage caused by polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene present in tobacco smoke [11]. However, *NQO1* also catalyzes the activation of certain procarcinogens found in tobacco smoke, including nitrosamines and heterocyclic amines [12], revealing the complex nature of the ROS pathway. A nonsynonymous C to T transition at nucleotide 609 of *NQO1* (rs1800566) results in a proline to serine amino acid substitution at codon 187, resulting in an enzyme with only 2% of the enzymatic activity of the wildtype protein [13]. Epidemiological studies have reported *NQO1* to be an important mediator of damage from ROS-generating carcinogens in tobacco smoke [10], but evidence for the role of this *NQO1* polymorphism in bladder cancer is conflicting.

Another enzyme, superoxide dismutase 2 (SOD2) plays a key role in protecting cells from oxidative injury [14]. A nonsynonymous C to T transition in exon 2 of the gene encoding for *SOD2* (rs4880) results in an alanine to valine amino acid substitution that is hypothesized to reduce enzyme activity [15] and thus leads to increased oxidative stress. However, results from studies on the functional significance of this polymorphism in bladder cancer are not clear [16].

To determine if functional polymorphisms in *NQO1* and *SOD2* genes can influence susceptibility to bladder cancer associated with tobacco smoking or SH infection, we examined data from our large case-control study of bladder cancer in Egypt.

2. Materials and methods

2.1. Study population

Cases were selected from 3 major referral centers in Egypt: the National Cancer Institute of Cairo University, the Oncology Center at Minia University, and the South Egypt Cancer Institute in Assiut. Noncancer controls were randomly selected from the general population and frequency matched to the cases by gender, 5-year age group, and region of residence (urban vs. rural), as previously reported in detail [6]. After informed consent was obtained, trained interviewers administered a detailed questionnaire that included questions on sociodemographic characteristics, smoking and environmental tobacco smoke (ETS), and medical history of schistosomiasis to both cases and controls. The protocol of the study was approved by the respective Institutional Review Boards of Georgetown University, Egypt's Ministry of Health, University of Maryland Baltimore, and the 3 medical centers in Egypt.

2.2. Case ascertainment

As reported earlier [6], the eligibility criteria for cases included primary bladder cancer diagnosis within 1 year of recruitment and age > 18 years. For each case, 1 of the 2 study pathologists examined available slides of urinary bladder tissue prepared from surgical or biopsy samples and reported the type of carcinoma as (1) urothelial cell carcinoma (UC), (2) SCC, (3) adenocarcinoma, or (4) other, including undifferentiated carcinomas. Carcinomas that metastasized to the bladder were excluded. We selected only cases that were confirmed by pathological examination to be either UC or SCC, the 2 predominant types of primary bladder cancer (95% of the cases).

2.3. Exposure assessment

The primary exposures of interest were tobacco use and SH infection. Participants who had smoked less than 100 cigarettes in their lifetime and had never smoked a water pipe were classified as “never users;” those who smoked less than 100 cigarettes in their lifetime but reported smoking water pipes only were classified as “water pipe only” users. “Cigarette only” users were those who had never smoked water pipes but had smoked at least 100 cigarettes in their lifetime, and “both water pipe and cigarette” users were those who reported smoking at least 100 cigarettes in their lifetime and had also used water pipes. History of schistosomiasis was based on the participants' self-report of whether or not they had been told about a diagnosis of schistosomiasis by their doctors. Exposure to ETS was also documented among all participants. ETS was assessed by asking each subject, whether a smoker or a nonsmoker, if they were exposed to other people's tobacco smoke at home only, outside the home only, or both at home and outside the home. The variable was dichotomized as exposed at either location or not exposed.

2.4. NQO1 and SOD2 genotyping

Blood samples were obtained from cases and controls in 10-ml collection tubes containing K_2EDTA and then processed to obtain the plasma and buffy coats. DNA was extracted from the buffy coats using standard methods. *NQO1 Pro187Ser* polymorphism (rs1800566) and *SOD2 Ala-9Val* polymorphism (rs4880) genotypes were determined using TaqMan allelic discrimination assays (Applied Biosystems) with a successful genotyping rate of 99% or higher and genotype concordance (among 10% blind quality-control duplicates) of > 99%.

2.5. Statistical analyses

Observed genotype distributions were tested for departure from the Hardy-Weinberg equilibrium (HWE) using the Pearson goodness of fit test. Logistic regression models were used to estimate the odds ratios (OR) and confidence intervals (CI) 95%. There were

sufficient numbers of subjects to examine each *SOD2* genotype separately in logistic regression models, whereas for *NQO1* we had to combine CT and TT owing to small numbers. All analyses were conducted using SAS software, version 9.2 (SAS Institute, Inc.).

3. Results

The present analyses include 902 cases and 804 controls for whom data on *NQO1* or *SOD2* genotypes, or both, were available. The characteristics of cases and controls are presented in Table 1. More cases than controls smoked and reported a history of schistosomiasis. The distribution of the genotypes for *SOD2* and *NQO1* were similar among cases and controls. Neither the *NQO1 Pro187Ser* polymorphism (rs1800566) nor the *SOD2 Ala-9Val* polymorphism (rs4880) violated the HWE assumption ($P > 0.05$ in all).

Using logistic conditions, we estimated the associations between bladder cancer and its major risk factors (tobacco use and schistosomiasis) among 3 different groups of individuals classified by *SOD2* genotype (CC, CT, and TT), after adjustment for gender, age, education, residence (urban vs. rural), and ETS. The results are shown in Table 2. When compared with participants with the CC or CT genotype, those with the TT genotype had a higher risk of cancer associated with cigarette use only or water pipe use only. This difference was even greater for participants reporting use of both cigarettes and water pipes. Conversely, history of SH infection was associated with greater odds of having bladder cancer among participants with CC genotype for *SOD2*. When we fit separate models for the 2 types of bladder cancer, we found that the magnitude of risk increase with smoking among those with the TT *SOD2* genotype was greater for the SCC type of bladder cancer (OR [CI 95%] = 6.37 [1.98–20.50]) than that for the UC type (OR [CI 95%] = 3.55 [1.33–9.44]).

Among participants with the TT or CT genotype for the *NQO1* enzyme, we observed similar trends, i.e. an increase in risk associated with smoking and decrease in risk associated with schistosomiasis, as compared with those with the CC genotype; however, these differences were much smaller than the effects observed with SOD2 and were not statistically significant (Table 3).

We also examined the effect of having combined “high-risk” genotypes (TT for *SOD2* and CT/TT for *NQO1*) on the tobacco use-bladder cancer and the SH infection-bladder cancer associations. When compared with participants with both “lowr-isk” genotypes (CC for both *SOD2* and *NQO1*) participants with both “high-risk” genotypes were at greater risk of bladder cancer if they reported tobacco use, but not if they reported a history of schistosomiasis (Table 4). The interaction between tobacco smoke exposure and genes was further supported by the results of the analyses we restricted to never smokers ($n = 580$); among never smokers with combined “low-risk” genotypes, the adjusted OR and 95% CI for being exposed to ETS was (OR [CI 95%] = 1.82 [0.46–7.20]); whereas among those with combined “high-risk” genotypes, the estimate was (OR [CI 95%] = 3.81 [1.10–13.20]).

4. Discussion

We observed evidence of gene-environment interactions between the endogenous genes for the antioxidant SOD2 and NQO1 enzymes and the common environmental risk factors for bladder cancer—tobacco use and SH infection. Water pipe and cigarette use were more strongly associated with cancer risk among participants with the TT genotype for *SOD2* as compared with those with the CC or the CT genotype; and to a much lesser extent similar associations were observed among those with CT/TT *NQO1* genotypes as compared with the CC genotype. However, among participants with the CC genotype that code for the most

active form of both enzymes, greater odds of having bladder cancer were associated with history of SH infection; the difference was significant for *SOD2* but not for *NQO1*.

To the best of our knowledge, this is the first study to evaluate the effects of *NQO1* and *SOD2* polymorphisms on bladder cancer risk associated with water pipe smoking. We have previously reported water pipe smoking, which is common in Middle Eastern and North African countries, to be an independent risk factor for bladder cancer risk after adjustment for other risk factors [6]. In the present study, we observed that the association of water pipe smoking with cancer risk was stronger among those with the TT genotype for *SOD2* and the CT/TT genotypes for *NQO1*. The pattern of OR in the stratified analysis (Tables 2 and 3) suggest that these genotypes play a potentially important role in modifying the effect of water pipe smoking on bladder cancer risk. Even ETS, which we have previously found to be an independent risk factor for bladder cancer [17], appears to interact with *SOD2* genotype in contributing to this malignancy. The wild-type isoform of SOD2 has been reported to have an increased relative level of superoxide scavenging capacity [15] and this might explain its associations with lesser risk from tobacco smoke.

Results from our study on the interaction between cigarette smoking and *SOD2* in bladder cancer risk support results from a previous study on Italian smokers that reported an increased bladder cancer risk associated with the *SOD2* TT genotype [9], but are not consistent with the findings reported in a study among Caucasian former smokers [18]. Possible reasons for these conflicting results in prior studies include small sample sizes, interethnic differences in allele frequency, poor matching between cases and controls, and publication bias. Allele frequencies among both cases and controls in our study were in HWE and the variant allele frequencies in these groups were similar to those reported in other studies.

The finding of a suggestive gene-environment interaction between cigarette smoking and *NQO1* in bladder cancer risk is supported by results from previous studies [10,19]. However, other studies have failed to find a significant association between the *NQO1* T allele and bladder cancer risk among smokers [9,18] or have reported, conversely, an increased risk associated with the wild-type C allele [20]. Possible reasons for these discrepancies might include differences in population stratification, genotyping methods, linkage disequilibrium, or residual confounding factors.

To the best of our knowledge, this is also the first study to evaluate the effect of *NQO1* and *SOD2* polymorphisms on bladder cancer risk associated with SH infection. We observed a statistically significant interaction between the *SOD2* genotype and SH infection in cancer risk. Participants with the *SOD2* CC genotype had an increased risk of bladder cancer associated with schistosomiasis as compared with those with the TT genotype. Recent reports suggest that SH infection is indeed a risk factor not only for SCC but also for UC in endemic areas, including Egypt [3,6]. SH has been classified as a class I carcinogen. Its primary mechanism of tumor development is thought to be the endogenous production of reactive oxygen or nitrogen species, or both, through oxidative stress, chronically generated by inflammation related to the deposition of vast numbers of SH eggs in the bladder subepithelial tissues [7]. Recent in vitro and animal experiments also suggest that SH infection down-regulates the expression of the estrogen receptor in the bladder [21]. This antiestrogenic activity is mediated by a number of estradiollike molecules expressed by the parasite and could be related to activation of signaling pathways related to cancer pathogenesis. This hypothesis is consistent with our previous findings of associations between bladder cancer risk in women and indicators of lifetime estrogen exposure [22].

The results of this study support the hypothesis that genes encoding the endogenous antioxidant enzymes *NQO1* and *SOD2* are potent modifiers of bladder cancer risk in smokers of both cigarettes and water pipes and among those exposed to ETS. Tobacco smoke from both cigarettes and water pipes is a rich source of ROS including the PAH benzo[*a*]pyrene, an International Agency for Research on Cancer Group I carcinogen, and superoxide anion radical [23]. It has long been known that several PAHs, including benzo[*a*]pyrene, can produce cancers in experimental animals, and epidemiological studies of exposed workers, especially in coke-oven and aluminum smelters, have shown excesses of bladder cancer [24]. Superoxide accumulates in the bladder and has also been shown to induce DNA damage and neoplasia [25]. *NQO1* protects cells from PAHs present in cigarette and water pipe smoke by catalyzing the reduction of quinones, including benzo[*a*] pyrene metabolites, to less toxic hydroquinones, thereby protecting the cell against cytotoxicity by reducing the cellular concentration of free quinone available for single-electron reduction [26]. In addition, *NQO1* directly scavenges the ROS superoxide and serves as a supplement enzyme system to *SOD2* for scavenging superoxide in cellular systems [27]. Finally, *NQO1* expression has been found to be elevated in bladder tumor tissue, where it is also known to play an important role in regulating p53 functions by inhibiting its degradation, thereby suggesting that lack of *NQO1* activity increases susceptibility to tumor development [28]. The variant T allele of the *NQO1* gene is associated with trace amounts of *NQO1* protein but no *NQO1* activity. Thus, individuals who carry the variant allele have greatly reduced or no *NQO1* detoxification activity and hence may be at a higher risk of tobacco-related bladder cancer.

SOD2 detoxifies superoxide radicals in mitochondria by converting anion superoxide to less toxic hydrogen peroxide and oxygen and thus plays a key role in protecting cells from oxidative stress. Although the functional significance of the *SOD2* rs4880 polymorphism is unclear, it has been suggested that the variant T allele disrupts the effective transport of the SOD2 enzyme into the mitochondria, thus inhibiting its ability to effectively scavenge superoxide [14,29]. Our findings further support the hypothesis that this polymorphism results in reduced ROS detoxification and hence an association with increased bladder cancer risk among smokers of cigarettes and water pipes.

The strengths of our study include a population-based control recruitment, high participation rates for both cases and controls, i.e., 84% and 97%, respectively [6], and case confirmation across recruitment centers by the same team of pathologists, thereby decreasing interobserver discrepancies. Our study also had several limitations. Data on *SOD2* and *NQO1* genotypes were not available for all cases and controls, which lead to concerns about selection bias. However, the characteristics of the present study subpopulation are quite similar to the parent study [6] in terms of never smokers (40% and 29%) and history of SH infection (55% and 45%), among controls and cases, respectively. This suggests minimal selection bias. There is no reliable biomarker to confirm history of schistosomiasis, so the study had to rely on self-reported data from both cases and controls, and thus a nondifferential bias. In addition, the lack of tobacco use among Egyptian women and the high prevalence of low-to-moderate level of cigarette smoking among Egyptian men might limit the generalizability of our findings to Western populations.

In summary, our findings suggest that common variations in genes coding for endogenous antioxidant *SOD2* and *NQO1* may modulate the association between tobacco smoking and bladder cancer risk. Individuals who possess the TT genotype of *SOD2* are at high risk of having bladder cancer whether they smoke water pipe or cigarette or were passively exposed to ETS. These findings have potentially important public health implications not only for Egypt, but worldwide, where the prevalence of water pipe smoking is increasing among youth who perceive it as a less harmful activity than cigarette smoking [30].

Acknowledgments

This work was supported by NCI Grant R01CA115618 (C.A.L.).

References

1. Ferlay, J.; Shin, HR.; Bray, F.; Forman, D.; Mathers, C.; Parkin, DM. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available at: http://globocan.iarc.fr.
2. Murta-Nascimento C, Silverman DT, Kogevinas M, García-Closas M, Rothman N, Tardón A, et al. Risk of bladder cancer associated with family history of cancer: do low-penetrance polymorphisms account for the increase in risk? Cancer Epidemiol Biomarkers Prev. 2007; 16:1595–1600. [PubMed: 17684133]
3. Felix AS, Soliman AS, Khaled H, Zaghloul MS, Banerjee M, El-Baradie M, et al. The changing patterns of bladder cancer in Egypt over the past 26 years. Cancer Causes Control. 2008; 19:421–429. [PubMed: 18188671]
4. Ibrahim AS, Khaled HM. Urinary bladder cancer. Freedman LS, Edwards BK, Ries LAG, Young JL, editors. A.S.Ibrahim. H.M. Khaled. Urinary bladder cancer. Scelo, Brennan P. The epidemiology of bladder and kidney cancer. Nat Clin Pract Urol. 2007; 4:205–217. [PubMed: 17415353]
6. Zheng YL, Amr S, Saleh DA, Dash C, Ezzat S, Mikhail NN, et al. Urinary bladder cancer risk factors in Egypt: a multicenter case-control study. Cancer Epidemiol Biomarkers Prev. 2012; 21:537–546. [PubMed: 22147365]
7. Rosin MP, Anwar WA, Ward AJ. Inflammation, chromosomal instability, and cancer: the schistosomiasis model. Cancer Res. 1994; 54:1929–1933.
8. Ohnishi S, Murata M, Kawanishi S. Oxidative DNA damage induced by a metabolite of 2-naphthylamine, a smoking-related bladder carcinogen. Jpn J Cancer Res. 2002; 93:736–743. [PubMed: 12149138]
9. Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F, et al. NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. Int J Cancer. 2004; 110:598–604. [PubMed: 15122594]
10. Park SJ, Zhao H, Spitz MR, Grossman HB, Wu X. An association between NQO1 genetic polymorphism and risk of bladder cancer. Mutat Res. 2003; 536:131–137. [PubMed: 12694753]
11. Joseph P, Jaiswal AK. NAD(P)H:quinone oxidoreductase1 (DT diaphorase) specifically prevents the formation of benzo[a]pyrene quinone-DNA adducts generated by cytochrome P4501A1 and P450 reductase. Proc Natl Acad Sci USA. 1994; 91:8413–8417. [PubMed: 8078896]
12. Chen S, Knox R, Lewis AD, Friedlos F, Workman P, Deng PS, et al. Catalytic properties of NAD(P)H:quinone acceptor oxidoreductase: study involving mouse, rat, human, and mouse-rat chimeric enzymes. Mol Pharmacol. 1995; 47:934–939. [PubMed: 7746280]
13. Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, et al. Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase). Br J Cancer. 1997; 75:69–75. [PubMed: 9000600]
14. Rosenblum JS, Gilula NB, Lerner RA. On signal sequence polymorphisms and diseases of distribution. Proc Natl Acad Sci USA. 1996; 93:4471–4473. [PubMed: 8633092]
15. Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, et al. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics. 2005; 15:311–319. [PubMed: 15864132]
16. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenet Genomics. 2006; 16:279–286. [PubMed: 16538174]
17. Amr S, Dawson R, Saleh DA, Magder L, Mikhail NN, St. George DM, et al. Agricultural workers and urinary bladder cancer risk in Egypt. Arch Environ Occup Health. :719556. [in press].

18. Vineis P, Veglia F, Garte S, Malaveille C, Matullo G, Dunning A, et al. Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol. 2007; 18:1230–1242. [PubMed: 17496311]
19. Wang YH, Lee YH, Tseng PT, Shen CH, Chiou HY. Human NAD(P) H:quinone oxidoreductase 1 (NQO1) and sulfotransferase 1A1 (SULT1A1) polymorphisms and urothelial cancer risk in Taiwan. J Cancer Res Clin Oncol. 2008; 134:203–209. [PubMed: 17619904]
20. Choi JY, Lee KM, Cho SH, Kim SW, Choi HY, Lee SY, et al. CYP2E1 and NQO1 genotypes, smoking and bladder cancer. Pharmacogenetics. 2003; 13:349–355. [PubMed: 12777965]
21. Botelho MC, Ribeiro R, Vale N, Oliveira P, Medeiros R, Lopes C, et al. Inactivation of estrogen receptor by Schistosoma haematobium total antigen in bladder urothelial cells. Oncol Rep. 2012; 27:356–362. [PubMed: 22089035]
22. Wolpert BJ, Amr S, Ezzat S, Saleh D, Gouda I, Loay I, et al. Estrogen exposure and bladder cancer risk in Egyptian women. Maturitas. 2010; 67:353–357. [PubMed: 20813471]
23. Pryor, WA.; Tamura, M.; Dooley, MM.; Premovic, P.; Hales, BJ.; Church, DF. Reactive oxy-radicals from cigarette smoke and their physiological effects. In: Greenwald, RA.; Cohen, G., editors. Oxy Radicals and Their Scavenger Systems: Cellular and Medical Aspects. Vol. Vol II. New York: Elsevier; 1983. p. 185-192.
24. Boffetta P, Jourenkova N, Gustavsson P. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control. 1997; 8:444–472. [PubMed: 9498904]
25. Pryor WA, Hales BJ, Premovic PI, Church DF. The radicals in cigarette tar: their nature and suggested physiological implications. Science. 1983; 220:425–427. [PubMed: 6301009]
26. Lind C, Cadenas E, Hochstein P, Ernster L. DT-diaphorase: purification, properties, and function. Methods Enzymol. 1990; 186:287–301. [PubMed: 2233301]
27. Siegel D, Gustafson DL, Dehn DL, Han JY, Boonchoong P, Berliner LJ, et al. NAD(P)H:quinone oxidoreductase 1: role as a superoxide scavenger. Mol Pharmacol. 2004; 65:1238–1247. [PubMed: 15102952]
28. Asher G, Lotem J, Cohen B, Sachs L, Shaul Y. Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1. Proc Natl Acad Sci USA. 2001; 98:1188–1193. [PubMed: 11158615]
29. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa- Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun. 1996; 226:561–565. [PubMed: 8806673]
30. Labib N, Radwan G, Mikhail N, Mohamed MK, Setouhy ME, Loffredo C, et al. Comparison of cigarette and water pipe smoking among female university students in Egypt. Nicotine Tob Res. 2007; 9:591–596. [PubMed: 17454715]

Table 1

Sociodemographic and genotype characteristics of bladder cancer cases and controls

Characteristic	Controls *n* = 804	Cases *n* = 902	*P* value
Age (mean ± SD), y	56.4 ± 12.0	58.5 ± 10.8	0.0001
Gender (%)			
F	213 (26.5)	174 (19.3)	0.0004
M	591 (73.5)	728 (80.7)	
Education (%)			
None	528 (65.7)	655 (72.7)	
Some	276 (34.3)	246 (27.3)	0.001
		1 missing	
History of schistosomiasis (%)			
No	465 (57.9)	392 (43.5)	
Yes	297 (36.9)	450 (49.9)	< 0.0001
Unknown	42 (5.2)	60 (6.6)	
Tobacco use (%)			
Never	327 (40.7)	263 (29.2)	< 0.0001
Ever	477 (59.3)	639 (70.8)	
Tobacco use (%)			
Never	327 (40.7)	263 (29.2)	
Water pipe only	78 (9.7)	87 (9.6)	< 0.0001
Cigarette only	352 (43.8)	434 (48.1)	
Both cigarette and water pipe	47 (5.8)	118 (13.1)	
ETS among never smokers (%)			
No	251 (76.8)	187 (71.1)	0.12
Yes	76 (23.2)	76 (28.9)	
SOD2 genotype (%)			
TT	242 (30.2)	272 (30.4)	
TC	369 (46.0)	413 (46.2)	0.97
CC	191 (23.8)	209 (23.4)	
Undetermined	2	8	
NQO1 genotype (%)			
TT	51 (6.4)	53 (5.9)	
TC	276 (34.6)	323 (36.1)	0.78
CC	470 (59.0)	519 (58.0)	
Undetermined	7	7	

SD = standard deviation.

Table 2

Association between bladder cancer and its risk factors among Egyptians, stratified by superoxide dismutase 2 *(SOD2)* genotypes

Risk factor	SOD2 genotype		
	CC ***n*** **= 382**	**TC or CT** ***n*** **= 766**	**TT** ***n*** **= 501**
Smoking			
Never	Ref.	Ref.	Ref.
Water pipe only	1.20 (0.47–3.11)[a]	1.08 (0.56–2.09)	2.43 (1.08–5.45)
Cigarette only	1.14 (0.57–2.26)	0.92 (0.55–1.55)	2.26 (1.22–4.20)
Both	2.56 (0.97–6.74)	1.43 (0.69–2.96)	4.41 (1.86–10.42)
Schistosomiasis history			
No	Ref.	Ref.	Ref.
Yes	3.59 (2.21–5.84)	1.86 (1.33–2.60)	1.81 (1.17–2.79)

[a]Odds ratio and (95% confidence interval) adjusted for age (continuous), education, residence (urban vs. rural), gender, environmental tobacco smoke, and for either history of schistosomiasis or smoking.

Table 3

Association between bladder cancer and its risk factors among Egyptians, stratified by NAD(P)H:quinone oxidoreductase *(NQO1)* genotypes

Risk factor	NQO1 genotype	
	CC ***n*** **= 965**	**TC or CT and TT**[b] ***n*** **= 681**
Smoking		
Never	Ref.	Ref.
Water pipe only	1.36 (0.78–2.38)[a]	1.64 (0.79–3.42)
Cigarette only	1.23 (0.80–1.90)	1.55 (0.90–2.68)
Both	2.41 (1.30–4.45)	2.61 (1.23–5.53)
Schistosomiasis history		
No	Ref.	Ref.
Yes	2.23 (1.64–3.02)	2.02 (1.41–2.87)

[a] Odds ratio and (95% confidence interval) adjusted for age (continuous), education, residence (urban vs. rural), gender, environmental tobacco smoke, and for either history of schistosomiasis or smoking.

[b] The number of observations with TT genotype was very small (Table 1), therefore we pooled them with the heterozygotes.

Table 4

Association between bladder cancer and its risk factors among Egyptians, stratified by combinations of genotypes for SOD2 and NQO1

Risk factor	Combined genotypes	
	NQO1 (CC) and SOD2 (CC) ***n*** **= 231**	**NQO1 TC/TT and SOD2 TT** ***n*** **= 202**
Smoking		
Never	Ref.	Ref.
Water pipe only	1.60 (0.50–5.12)[a]	1.98 (0.87–4.48)
Cigarette only	0.96 (0.41–2.25)	1.49 (0.81–2.74)
Both	2.75 (0.77–9.77)	2.43 (1.03–5.77)
Schistosomiasis history		
No	Ref.	Ref.
Yes	4.41 (2.32–8.38)	1.85 (1.24–2.77)

[a]Odds ratio and (95% confidence interval) adjusted for age (continuous), education, residence (urban vs. rural), gender, environmental tobacco smoke, and for either history of schistosomiasis or smoking.