Polycyclic Aromatic Hydrocarbon-induced Oxidative Stress, Antioxidant Capacity, and the Risk of Lung Cancer: A Pilot Nested Case-control Study

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Abstract. *Aim: We conducted a pilot nested case−control study to prospectively evaluate the effects of polycyclic aromatic hydrocarbons (PAH) exposure, antioxidant capacity, and oxidative stress on lung carcinogenesis. Materials and Methods: Thirty-five patients with lung cancer and 140 age- and sex-matched controls were selected from a sub-cohort of the Korean Multi-center Cancer Cohort. PAH metabolites (1-hydroxypyrene and 2-naphthol), oxidative stress markers, and total antioxidant capacity (TAC) were assessed using urine samples collected at baseline. Results: The levels of urinary PAH metabolites and oxidative stress were not different between cases and controls. Urinary 1-hydroxypyrene and 2-naphthol levels were significantly associated with urinary oxidative stress markers only in lung cancer cases. Individuals with low urinary TAC and high urinary oxidative stress levels had significantly higher risk of lung cancer compared to those with high urinary TAC and low urinary oxidative stress levels. Conclusion: Oxidative stress induced by PAH exposure and TAC may be important determinants for the susceptibility to lung cancer.*

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Key Words: Polycyclic aromatic hydrocarbon, oxidative stress, antioxidant capacity, lung cancer, nested case–control study, 1-hydroxypyrene, thiobarbituric acid, 8-hydroxydeoxyguanosine, cotinine, 2-naphthol.

Lung cancer is the most common cancer and the leading cause of cancer-related deaths worldwide (1). In Korea, lung cancer is the fourth most prevalent type of cancer, and the mortality is the highest among all cancer types (2). Numerous epidemiological studies have suggested that oxidative stress plays a pivotal role in the carcinogenic mechanism of lung cancer (3-9). Free radicals and reactive oxygen species (ROS) are not only present in tobacco smoke, the most important cause of lung cancer, but are also generated during metabolism of polycyclic aromatic hydrocarbons (PAHs) found in smoke, which can cause severe damage to cellular macromolecules (*e.g.* DNA, proteins, and lipids) *via* oxidative stress (10).

Several prospective cohort studies have shown that increased dietary intake and pre-diagnostic blood levels of antioxidants were associated with a reduced risk of lung cancer (11, 12). Antioxidant defense mechanisms protect against free radical-induced oxidative stress, although their depletion does not necessarily mean that oxidative damage has occurred (10). Antioxidant defenses comprise of endogenous and exogenous antioxidants. Endogenous antioxidants include antioxidant enzymes and non-enzymatic antioxidants, such as glutathione, bilirubin, α-keto acids, melatonin, and lipoic acid, which are synthesized *in vivo*. Exogenous antioxidants are mainly derived from the diet and consist of ascorbic acid, vitamin E, carotenoids, and polyphenols. Inter-individual variations in antioxidant defense capacity are determined by various factors including age, diet, and genetic background, and these variations can affect the susceptibility to cancer (10, 13). The assay of total antioxidant capacity (TAC) has been used to measure the antioxidant capacity of a number of heterogeneous compounds with antioxidant activity in body fluids and thus may help evaluate the overall antioxidant status (10).

Tobacco smoke contains over 3,500 different chemical compounds, and at least 55 of these chemical compounds are known pulmonary carcinogens, such as PAHs, N-nitrosamines, aromatic amines, heterocyclic aromatic amines, aldehydes, arsenic, chromium, and nickel (3, 12, 14). Among them, PAHs are the most extensively studied pulmonary carcinogens, and their pathways of metabolic activation and detoxification have been well-determined (3). Absorbed PAHs are usually metabolically activated by cytochrome *P450* enzymes into highly reactive intermediates such as bay-region diol-epoxides, radical-cation intermediates, and PAH quinones (15, 16). These intermediates are transformed by hydroxylation and phase II enzymes into water-soluble metabolites that are more readily excreted in the urine (3, 15). Urinary 1-hydroxypyrene (1-OHP) and 2-naphthol have been used as biological markers of exposure to PAHs, and these two metabolites reflect various routes of exposure to PAHs, including not only tobacco smoking but also occupational exposure, air pollution, and food consumption (17, 18). Because PAH metabolites have a short half-life (<24 h) (19), the effects of PAH exposure on lung carcinogenesis need to be prospectively evaluated. However, to date, the causal relationship based on prospective studies has not been characterized.

In this pilot study, we prospectively investigated the urinary levels of PAH exposure, oxidative stress, TAC and their interrelationships in lung carcinogenesis.

Materials and Methods

Study subjects. For this nested case–control study, we selected 35 lung cancer cases and 140 controls from the Chungju Regional Cohort, which is a subcohort of the Korean Multi-center Cancer Cohort (KMCC), a multicenter prospective cohort designed to investigate the relationship between environmental factors, lifestyle factors, host factors, and the risk of cancers in Korea (20). The lung cancer cases were ascertained based on the record of linkage between the National Cancer Registry and the National Death Certificate System (20). Four healthy controls were matched to each case by age at the baseline, and gender. For the baseline survey, the trained personnel interviewed the study subjects, who have provided written informed consent, using a structured questionnaire including questions on demographic factors, smoking habits, alcohol consumption, and occupational history. Peripheral blood and urine samples were collected from all the subjects at the baseline of the cohort study (1996-2000). This study was approved by the Institutional Review Boards of Chungbuk National University Hospital, Korea (IRB no. 2011-09-072).

Analysis of urinary 1-OHP and 2-naphthol levels. Urinary 1-OHP and 2-naphthol levels were determined using a high-performance liquid chromatographic (HPLC) system with a fluorescence detector (21, 22). Urine samples were buffered with 0.2 M sodium acetate (pH 5.0) and hydrolyzed enzymatically using β-glucuronidase with sulfatase activity (G-0876; Sigma, St. Louis, MO, USA), for 16 h at 37˚C in a shaking water bath. After hydrolysis, acetonitrile was added, and the samples were centrifuged at 1000 ×*g* for 10 min. About 10 μl of the supernatant was injected into the HPLC system, which consisted of a pump (L-2130; Hitachi, Tokyo, Japan), an automatic injector (L-7200; Hitachi), a fluorescence detector (L7485; Hitachi), and a data acquisition module (Autochro-200; Younglin, Seoul, Korea). The columns used were a 150 mm reverse-phase column (TSK-GEL ODS-80TM; Tosoh, Tokyo, Japan) for 1-OHP and a 250 mm reverse-phase column (J'sphere ODS-H80; YMC, Wilmington, MA, USA) for 2-naphthol. The mobile phases used were acetonitrile:water (60:40) for 1-OHP and acetonitrile:water (38:62) for 2-naphthol, at a flow-rate of 1 ml/min. The excitation/emission wavelengths were 242/388 nm for 1-OHP and 227/355 nm for 2-naphthol. The urinary 1-OHP and 2-naphthol concentrations were adjusted to the urinary creatinine concentration (μg/g creatinine) to control for the variability in urine dilution.

Analysis of biomarkers for oxidative stress. 8-Hydroxydeoxyguanosine: The level of 8-hydroxydeoxyguanosine (8-OHdG) was measured using an 8-OHdG enzyme linked immunosorbent assay (ELISA) kit (8-OHdG Check; Japan Institute for the Control of Aging, Fukuroi, Japan). Briefly, urine samples were centrifuged, and 50 μl of the supernatant and 50 μl of an aliquot of the primary antibody were added to an 8-OHdG precoated microplate and incubated at 37˚C for 1 h. The plate was washed three times with phosphate-buffered saline. Horseradish peroxidase (HRP)-conjugated secondary antibody was added to each well, incubated at 37˚C for 1 h, and subsequently washed three times. A 100 μl enzyme substrate containing 3,3',5,5'-tetra-methyl-benzidine (TMB) was added, and the plates were incubated at room temperature for 15 min under dark conditions. The reaction was terminated by adding of 1 M phosphoric acid, and the absorbance at 450 nm was measured using a microplate reader (GENios; TECAN, Grödig/Salzburg, Austria). The concentration of 8-OHdG was calculated using a standard curve.

Thiobarbituric acid reactive substances: Urinary thiobarbituric acid reactive substances (TBARS) were determined using the HPLC system with a fluorescence detector (23). Briefly, 50 μl of 0.05% butylated hydroxytoluene (BHT), 150 μl of 0.1125 N nitric acid, and 150 μl of 42 mM thiobarbituric acid (TBA) were added to a 50 μl aliquot of the urine sample or 50 μl of 1,1,3,3-tetramethoxypropane (TMP) standard and mixed using a vortex mixer. Then, the samples were heated on a heat block (100˚C) for 1 h and then placed in ice-water for 5 min to cool; 300 μl of n-butanol was subsequently added for the extraction of TBARS, and then the samples were centrifuged at 10,000 × *g* for 5 min. Ten μl of the supernatant was injected into the HPLC system, which consisted of a pump (Lsp 930; Younglin), an automatic injector (SIL 10Avp; Shimadzu, Kyoto, Japan), a fluorescence detector (RF-10AxL; Shimadzu), and a data acquisition module (Autochro-200; Younglin). The columns used were a 150 mm reverse-phase column (TSK-GEL ODS-80TM; Tosoh), and the mobile phases used were potassium dihydrogen phosphate:methanol:acetonitrile (60:25:15, v/v/v), at a flow-rate of 1 ml/min. The excitation/emission wavelengths were 515/553 nm.

Analysis of urinary cotinine. The levels of cotinine were measured using a cotinine direct ELISA kit (Cotinine Direct Elisa; Calbiotech, Spring Vally, CA, USA) to represent the exposure level to tobacco smoke. Briefly, 10 μl of urine and 100 μl of HRP-conjugated cotinine were added to a microplate coated with a polyclonal antibody to cotinine and incubated at room temperature for 1 h under dark

Table I. *General characteristics and urinary biomarker levels of the lung cancer cases and controls.*

Variable	Cases	Controls	*p*-Value
N	35	140	
Age, years, mean±SD	68.87±6.86	68.86±6.85	0.991
Gender, N (%)			0.930
Male	27 (77.1)	108 (77.1)	
Female	8 (22.9)	32 (22.9)	
Height, cm, mean±SD	159.20±7.15	161.84±7.23	0.189
Weight, kg, mean±SD	59.46±10.05	58.89±7.40	0.834
Body mass index, kg/m^2, mean±SD	23.40±3.22	22.46±2.29	0.274
Smoking status, N (%)			<0.1
Never-smokers	7 (20.0)	48 (34.5)	
Current or ex- smokers	28 (80.0)	91 (65.5)	
Cumulative smoking amounts, N (%)			<0.05
<20 packs/year	11 (31.4)	71 (50.7)	
20–39 packs/year	9 (25.7)	28 (20.0)	
≥40 packs/year	15 (42.9)	41 (29.3)	
Alcohol drinking, N (%)			0.146
Non-drinkers	17 (48.6)	49 (35.2)	
Drinkers	18 (51.4)	90 (64.8)	
Urinary parameters*			
Cotinine, μg/g creatinine	93.37 (44.39-196.43)	69.31 (48.57-98.81)	0.448
2-Naphthol, μg/g creatinine	3.89 (2.38-6.37)	3.49 (2.83-4.29)	0.646
1-OHP, μg/g creatinine	0.06 (0.02-0.17)	0.04 (0.03-0.07)	0.670
TAC, mM UA equiv./mM creatinine	0.16 (0.12-0.23)	0.22 (0.19-0.25)	<0.1
TBARS, μmol/g creatinine	0.71 (0.44-1.16)	0.62 (0.49-0.78)	0.558
8-OHdG, μg/g creatinine	5.54 (4.57-6.73)	4.88 (4.43-5.38)	0.234

1-OHP: 1-hydroxypyrene; TAC: total antioxidant capacity; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxydeoxyguanosine. *Data are presented as geometric mean values (95% confidence intervals).

conditions. The plate was washed six times with distilled water. A 100 μl enzyme substrate containing TMB was added, and the plates were incubated at room temperature for 30 min under dark conditions. The reaction was terminated by adding the stop solution, and the absorbance at 450 nm was measured using a microplate reader (GENios; TECAN). The concentration of cotinine was calculated using a standard curve and was expressed as μg/g creatinine.

Analysis of urinary total antioxidant capacity. Urinary TAC was determined by the copper (II) reduction assay with bathocuproinedisulfonic acid disodium salt as the chelating agent (CUPRAC–BCS assay) (24, 25). Briefly, urine samples were diluted eight-fold with phosphate-buffered saline, and the diluted urine samples or the standard, were mixed with 0.25 mM BCS in test tubes. Then, 200 μl of the mixture was added to a microplate in duplicate, and absorbance at 490 nm was measured using a microplate reader. After the addition of 50 μl of 0.5 mM $CuSO_4$, the reaction mixture was incubated for 3 min at room temperature before adding 50 μl of 0.01 M EDTA to each well to terminate the reaction. The absorbance at 490 nm was measured. TAC was calculated by comparing the net absorbance for the samples to a standard curve obtained with uric acid (UA). Because the level of UA, which is a non-enzymatic antioxidant in urine, is higher in individuals who perform intense physical exercises and patients with arterial hypertension, metabolic syndrome, or kidney diseases (25, 26), the final TAC level in this study was adjusted to the relative contribution of UA by using the method proposed by Campos *et al*. (25).

Statistical analysis. All statistical analyses were performed using SPSS 19 (IBM, Armonk, NY, USA). The concentration of biomarkers for PAH exposure, oxidative stress, and TAC in the urine were log-transformed for the statistical analyses because they were not normally distributed. Differences in the categorical variables between cases and controls were tested using a chi-square test. Differences in the means between cases and controls were assessed using Student's *t*-test or Wilcoxon rank sum test. Spearman correlations between the urinary biomarkers were statistically tested with respect to group and smoking status. A general linear model including age, sex, smoking history, and levels of PAH exposure or TAC was used to determine the effects of the level of PAH exposure or TAC on oxidative stress. Finally, associations between lung cancer and the putative risk factors were estimated using odds ratios (ORs) and their corresponding 95% confidence intervals (95% CI) calculated using exact conditional logistic regression models, after adjusting for potential confounding factors such as age, sex, and smoking history. The level of statistical significance was set at $p<0.05$.

Results

There were no statistically significant differences between lung cancer cases and controls with respect to age, gender, height, weight, and body-mass index. The cumulative smoking amount was significantly different between the lung cancer cases and controls. Although not significant,

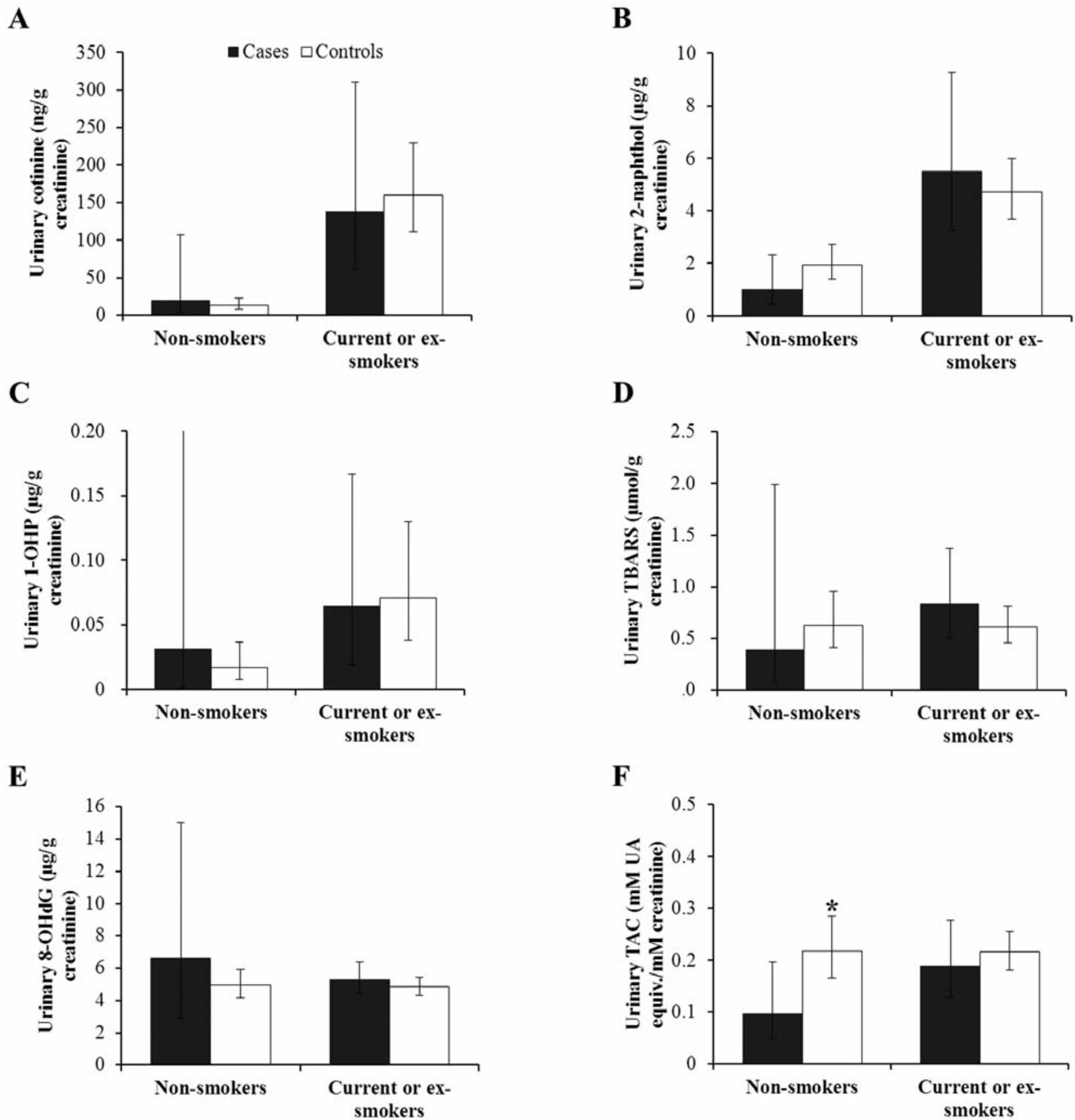

Figure 1. *Geometric means and 95% confidence intervals for urinary biomarkers of exposure (A: cotinine, B: 2-naphthol, C: 1-hydroxypyrene), oxidative stress markers (D: thiobarbituric acid reactive substances, E: 8-hydroxydeoxyguanosine), and total antioxidant capacity (F) in never-smokers and current or ex-smokers. An asterisk indicates a significant difference between the cases and controls according to the Wilcoxon rank sum test ($p<0.05$).*

the geometric means for urinary cotinine, 2-naphthol, 1-OHP, TBARS, and 8-OHdG were higher in lung cancer cases than in controls, while urinary TAC was lower in lung cancer cases than in controls, with a marginal significance (Table I).

Figure 1 shows the levels of urinary biomarkers of PAH exposure, oxidative stress markers, and TAC in the lung cancer cases and controls, according to the smoking status. The geometric means of urinary exposure (cotinine, 2-naphthol, and 1-OHP) and oxidative stress (TBARS and 8-OHdG)

Table II. *Spearman correlation coefficients between biomarkers of exposure and total antioxidant capacity (TAC) and oxidative stress markers.*

	Correlation coefficients				
	Cotinine	2-Naphthol	1-OHP	TAC	8-OHdG
With TBARS					
Cases					
All	0.169	0.220	0.290†	0.167	0.418*
Never-smokers	0.500	−0.214	0.000	−0.786*	0.679†
Current or ex-smokers	−0.004	0.186	0.297	0.221	0.414*
Controls					
All	0.067	−0.047	0.104	−0.009	0.178*
Never-smokers	0.139	−0.000	−0.074	0.064	0.072
Current or ex-smokers	0.012	−0.080	0.177	−0.038	0.246*
With 8-OHdG					
Cases					
All	0.255	0.261	0.158	−0.020	-
Never-smokers	0.607	0.321	0.214	−0.857*	-
Current or ex-smokers	0.274	0.423*	0.201	0.281	-
Controls					
All	0.084	0.067	0.003	−0.020	-
Never-smokers	0.003	0.109	0.024	−0.162	-
Current or ex-smokers	0.160	0.075	0.025	0.050	-

1-OHP: 1-hydroxypyrene; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxydeoxyguanosine. *p<0.05, †p<0.1.

markers were not significantly different between the lung cancer cases and controls neither in never-smokers nor current or ex-smokers. However, among never-smokers, the urinary TAC level was significantly lower in the cases (0.096 mM UA equiv./mM creatinine) than in the controls (0.188 mM UA equiv./mM creatinine).

The levels of urinary cotinine and PAHs exposure markers (1-OHP and 2-naphthol) were significantly lower in non-smokers than in current or ex-smokers. Those significant differences were found not only in comparisons within the lung cancer cases but also in those within the controls. TBARS, 8-OHdG and TAC levels, however, were not significantly different according to the smoking status.

In the correlation analyses between the markers of PAH exposure (1-OHP and 2-naphthol) and TAC, and urinary oxidative stress markers (8-OHdG and TBARS), Spearman's correlation coefficient between urinary 2-naphthol and 8-OHdG level was statistically significant only in current or ex-smoker lung cancer cases (r=0.423, p<0.05). Urinary 1-OHP was marginally and significantly correlated with urinary TBARS in the lung cancer cases overall (r=0.290, p<0.1). Among the never-smoker lung cancer cases, the urinary TAC level was negatively correlated with both urinary TBARS and 8-OHdG levels. We did not find any suggestive relationships among controls (Table II).

In the general linear model adjusting for age, sex, and smoking status, urinary 1-OHP was significantly associated with the urinary TBARS levels in the lung cancer cases. In addition, the urinary level of cotinine and 2-naphthol were related to the urinary 8-OHdG level in the lung cancer cases. However, for the control group, there were no variables correlated with the levels of oxidative stress markers (Table III).

Table IV shows the results for lung cancer risk according to PAH exposure, TAC, oxidative stress, and their combinations. Although not statistically significant, high urinary cotinine, 2-naphthol, 1-OHP, TBARS, and 8-OHdG levels were associated with an increased risk of lung cancer, while a high urinary TAC level was related to a reduced lung cancer risk. Interestingly, the combination of both high urinary 1-OHP and 8-OHdG levels was associated with an increased risk for lung cancer with a marginal significance (OR=2.73, 95% CI=0.94-8.78). Lung cancer risk increased significantly for the combination of low urinary TAC and high urinary 8-OHdG levels than in that of high urinary TAC and low urinary 8-OHdG levels (OR=4.80, 95% CI=1.06-32.17).

Discussion

The results of the present pilot study suggest that PAH exposure is associated with an increase in oxidative stress in the body and that individuals with low TAC and high oxidative DNA damage have a significantly higher risk of lung cancer compared to individuals with high TAC and low oxidative DNA damage. Previous studies similarly, have

reported results that increased oxidative stress and lower TAC have an effect on the incidence of lung cancer (6-8). However, these studies did not suggest a clear causal relationship because they used biological samples collected after lung cancer diagnosis. Since in the present study, a prospective study design was used to evaluate PAH exposure, oxidative stress, and TAC, there is no temporal ambiguity between the exposure to risk factors and the incidence of lung cancer in our results.

In this study, we assessed the exposure to PAHs by measuring the urinary concentration of 2-naphthol, a metabolite of naphthalene, and 1-OHP, a metabolite of pyrene. Absorbed naphthalene is metabolized into the reactive metabolites naphthalene-1,2-oxide (NPO), 1,2-naphthoquinone (1,2-NPQ), and 1,4-naphthoquinone (1,4-NPQ); these electrophiles can covalently bond with macromolecules, including DNA and proteins (27). It was reported that PAH-*o*-quinone produced more than 60 8-OHdG adducts per 10^5dG in salmon testis DNA, and the naphthalene metabolite 1,2-NPQ was shown to have a relatively high 8-OHdG production rate than the other PAH-*o*-quinones (15). Similarly, our study also showed that an increase in urinary 2-naphthol concentration among lung cancer cases was linked to a significant increase in urinary 8-OHdG, a marker of oxidative DNA damage, after adjusting for age, gender, and smoking status. In addition, PAH easily penetrates biological membranes because of its lipophilic character, and ROS, such as hydroxyl radicals, produced from PAH metabolism can lead to the peroxidation of polyunsaturated lipids, which are the major constituent of cell membranes (9). Our results also indicate that the urinary level of 1-OHP of lung cancer cases has a significant association with urinary concentration of TBARS, a lipid peroxidation marker, and this finding was consistent with the studies conducted by Pan *et al*. (28) and Jeng *et al*. (29). This shows that oxidative stress in the tissues and lipid peroxidation in the membranes increase with PAH exposure.

In the present study, PAH exposure levels were similar to those reported in previous studies for South Koreans without occupational exposure (17, 30, 31), and there was no difference in PAH levels between lung cancer cases and controls. However, a significant increase in oxidative stress due to PAH exposure was observed only in the lung cancer cases. This finding indicates that there may be a difference in the sensitivity of the two groups to PAH exposure, and there are several possible factors that may account for that difference. Firstly, there may be a difference between the two groups with respect to the quantity of reactive substances produced in PAH metabolism, because of polymorphisms in the metabolic enzymes involved in the biotransformation and detoxification of PAHs (32). Secondly, there may be differences in antioxidant capacity, which is a defense mechanism against oxidative damage inside the body. In the present study, there was indeed a slight difference in urinary TAC measurements between the two groups (lung cancer cases 0.16 *vs*. controls 0.22, $p<0.1$). This means that the level of endogenous production or dietary intake of non-enzymatic antioxidants was relatively lower in the lung-cancer-cases group before the incidence of cancer. Thirdly, in the lung cancer group, simultaneous exposure to PAHs and other carcinogens, such as heavy metals, could have led to a synergistic effect among those carcinogens such that oxidative stress and the risk of cancer incidence increased concurrently (33).

Table III. *General linear models for urinary oxidative stress markers in lung cancer cases and controls.*

Dependent variable	Independent variable	Cases		Controls	
		Beta	*p*-Value	Beta	*p*-Value
TBARS	Age	0.029	0.434	0.028	0.105
	Gender	0.885	0.321	0.222	0.576
	Smoking status	0.254	0.790	0.368	0.351
	Urinary cotinine	0.166	0.174	0.089	0.215
	Age	0.032	0.431	0.027	0.119
	Gender	0.771	0.481	0.159	0.691
	Smoking status	0.03	0.980	–0.005	0.990
	Urinary 2-naphthol	0.116	0.580	–0.129	0.231
	Age	0.039	0.268	0.024	0.169
	Gender	0.612	0.475	0.172	0.672
	Smoking status	–0.173	0.844	0.151	0.667
	Urinary 1-OHP	0.151	<0.05	0.023	0.619
	Age	0.036	0.320	0.027	0.114
	Gender	1.118	0.228	0.164	0.590
	Smoking status	0.356	0.717	0.136	0.639
	Urinary TAC	0.364	0.177	0.133	0.372
8-OHdG	Age	0.034	<0.01	–0.008	0.277
	Gender	0.553	<0.1	–0.127	0.452
	Smoking status	0.877	<0.05	–0.018	0.915
	Urinary cotinine	0.118	<0.01	0.011	0.710
	Age	0.035	<0.05	–0.008	0.258
	Gender	0.561	0.137	–0.123	0.467
	Smoking status	0.949	<0.05	–0.033	0.835
	Urinary 2-naphthol	0.174	<0.05	0.012	0.788
	Age	0.039	<0.01	–0.008	0.293
	Gender	0.425	0.194	–0.121	0.480
	Smoking status	0.581	<0.1	–0.049	0.744
	Urinary 1-OHP	0.048	<0.1	–0.004	0.825
	Age	0.036	<0.05	–0.008	0.293
	Gender	0.540	0.128	–0.103	0.717
	Smoking status	0.681	<0.1	–0.033	0.968
	Urinary TAC	0.067	0.510	–0.071	0.245

1-OHP: 1-hydroxypyrene; TAC: total antioxidant capacity; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxydeoxyguanosine.

Our results show that an association between total antioxidant capacity and urinary oxidative stress levels in the lung cancer group yielded opposite results by the smoking status. In never-smokers among lung cancer cases, oxidative

Table IV. *Odds ratios (ORs) for lung cancer according to urinary biomarker levels* and their combinations.*

Variable	N (%)		OR† (95% CI)	*p*-Value
	Cases	Controls		
Cotinine (μg/g creatinine)				
Low (<123.74)	14 (40.0)	76 (54.3)	1.00	
High (≥123.74)	21 (60.0)	64 (45.7)	1.32 (0.52, 3.44)	0.676
2-Naphthol (μg/g creatinine)				
Low (<4.27)	14 (40.0)	76 (54.3)	1.00	
High (≥4.27)	21 (60.0)	64 (45.7)	1.39 (0.58, 3.44)	0.553
1-OHP (μg/g creatinine)				
Low (<0.10)	14 (40.0)	77 (55.0)	1.00	
High (≥0.10)	21 (60.0)	63 (45.0)	1.70 (0.74, 4.03)	0.242
TAC (mM UA equiv./mM creatinine)				
Low (<0.24)	23 (65.7)	76 (54.3)	1.00	
High (≥0.24)	12 (34.3)	64 (45.7)	0.60 (0.23, 1.46)	0.311
TBARS (μmol/g creatinine)				
Low (<0.75)	14 (40.0)	76 (54.3)	1.00	
High (≥0.75)	21 (60.0)	64 (45.7)	1.83 (0.78, 4.41)	0.182
8-OHdG (μg/g creatinine)				
Low (<4.84)	12 (34.3)	76 (54.3)	1.00	
High (≥4.84)	23 (65.7)	64 (45.7)	2.00 (0.87, 4.78)	0.109.
Combination of 1-OHP and 8-OHdG				
Both low	8 (22.9)	43 (30.7)	1.00	
Other combinations	10 (28.6)	67 (47.9)	0.62 (0.19, 1.99)	0.505
Both high	17 (48.6)	30 (21.4)	2.73 (0.94, 8.78)	<0.1
Combination of TAC and 8-OHdG				
High TAC and low 8-OHdG	3 (8.6)	34 (24.3)	1.00	
Other combinations	18 (51.4)	72 (51.4)	3.43 (0.76, 21.89)	0.130
Low TAC and high 8-OHdG	14 (40.0)	34 (24.3)	4.80 (1.06, 32.17)	<0.05
Combination of 1-OHP, 8-OHdG, and TAC				
Low 1-OHP and 8-OHdG and high TAC	1 (2.3)	13 (8.9)	1.00	
Other combinations	25 (71.4)	119 (82.1)	2.62 (0.37, 114.03)	0.603
High 1-OHP and 8-OHdG and low TAC	9 (25.7)	13 (9.0)	8.01 (0.91, 391.14)	<0.1

1-OHP: 1-hydroxypyrene; TAC: total antioxidant capacity; TBARS: thiobarbituric acid reactive substances; 8-OHdG: 8-hydroxydeoxyguanosine. *Urinary biomarker levels were divided into low and high groups based on the median value of the controls. †Adjusted for age, gender, and smoking history.

stress significantly decreased as urinary TAC increased, however, the two markers showed a positive correlation in current or ex-smokers, but this was not statistically significant. In addition, the TAC of never-smokers among lung cancer cases was half that of smokers among lung cancer cases. It is thought that large amounts of ROS are produced in smokers and the antioxidant capacity as a defensive mechanism also increases by smoking, thus accounting for the positive correlation between the two markers. However, since never-smokers have a lower antioxidant capacity, inadequate detoxification of ROS may have resulted in high levels of oxidative stress (34, 35). Similarly to our results, the serum antioxidant capacity (AOC) was significantly lower in patients with non-small cell lung cancer who were never-smokers than in smokers, and that oxidative DNA damage in the lung tissues of the never-smokers with low AOC was increased in comparison with that of patients with benign lung disease (7). Although never-smokers among lung cancer cases have lower levels of oxidative stress than current or ex-smokers, if they have also weaker defense mechanisms against oxidative stress, resulting in greater oxidative damage and increased risk of cancer incidence. Thus, it can be suggested that never-smokers among lung cancer cases may have developed lung cancer upon exposure to carcinogens other than those found in tobacco smoke or have a very high genetic susceptibility to cancer.

Although this pilot study has limited statistical power due to the small sample size, to date, there have been no prospective epidemiological studies that simultaneously evaluate PAH exposure, oxidative stress, and TAC with respect to lung cancer. In the present study, individuals with high urinary 1-OHP and 8-OHdG levels had a 2.7-fold increase of lung cancer risk compared to individuals with

low urinary 1-OHP and 8-OHdG levels. This implies that the risk of lung cancer is significantly higher in individuals who show an increase in oxidative stress upon increased smoking or PAH exposure. Individuals with low urinary TAC and high urinary 8-OHdG levels had a 4.8-fold increase in lung cancer risk than individuals with high urinary TAC and low urinary 8-OHdG levels. In addition, when all three markers were combined, the OR for lung cancer incidence was as high as 8.01 (95% CI=0.91–391.14, p=0.066) for individuals with high urinary 1-OHP and 8-OHdG levels and low urinary TAC, in comparison with that of individuals with low urinary 1-OHP and 8-OHdG levels and high urinary TAC. This result shows that high PAH exposure, oxidative stress, and a decrease in TAC can synergistically increase lung cancer risk.

The results of the present pilot study indicate that individuals whose oxidative stress increases concurrently with increased exposure to smoking or PAHs are more likely to develop lung cancer. Out of these individuals, those with low baseline TAC are especially susceptible to lung cancer. Additional studies with larger sample sizes are warranted to confirm the results from this study.

Conflicts of Interest

None declared.

Acknowledgements

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (1120330).

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Received May 15, 2013
Revised June 14, 2013
Accepted June 17, 2013