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Urinary pH, cigarette smoking and bladder cancer risk

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Glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolite can be rapidly hydrolyzed in acidic urine to undergo further metabolic activation and form DNA adducts in the urothelium. We conducted a large multicenter case–control study in Spain to explore the etiology of bladder cancer and evaluated the association between urine pH and bladder cancer risk, alone and in combination with cigarette smoking. In total, 712 incident urothelial cell carcinoma cases and 611 hospital controls directly measured their urine pH with dipsticks twice a day (first void in the morning and early in the evening) during four consecutive days 2 weeks after hospital discharge. We found that a consistently acidic urine pH ≤6.0 was associated with an increased risk of bladder cancer [odds ratio (OR) = 1.5, 95% confidence interval (CI): 1.2–1.9] compared with all other subjects. Furthermore, risk estimates for smoking intensity and risk of bladder cancer among current smokers tended to be higher for those with a consistently acidic urine (OR = 8.8, 11.5 and 23.8) compared with those without (OR = 4.3, 7.7 and 5.8, respectively, for 1–19, 20–29 and 30+ cigarettes per day; $P_{interaction}$ for 30+ cigarettes per day = 0.024). These results suggest that urine pH, which is determined primarily by diet and body surface area, may be an important modifier of smoking and risk of bladder cancer.

Introduction

Aromatic amines are well-known bladder carcinogens and urine pH can strongly influence their presence in the urine as free aromatic amine compounds (1). For example, toxicokinetic studies have shown that *N*-glucuronide of *N*-acetyl-benzidine (ABZ), which is synthesized in the liver, is excreted into the urinary bladder where it is relatively stable under neutral pH conditions. However, in the presence of acidic conditions, it is rapidly hydrolyzed and can undergo further metabolism to bind to DNA (2,3). In a study of workers exposed to benzidine and benzidine-based dyes, urine pH <6.0 was associated with 10-fold higher levels of exfoliated urothelial cell DNA adduct levels (4). There is also *in vitro* evidence that urine pH has a similar effect on aromatic amines derived from cigarette smoke such as 4-aminobiphenyl (4-ABP) and its metabolite *N*-hydroxy-4-aminobyphenyl (N-OH-4-ABP) (5). For example, the half-life of 4-ABP *N*-glucuronide conjugates, before being hydrolyzed, is 11 min at pH 5.5 and 37°C compared with >3 h at pH 7.4 (6). Furthermore, a toxicokinetic study showed that urine pH was a strong contributor to interindividual variation in DNA binding of ABP in the human bladder (7).

To date, no study has reported an association between directly measured urinary pH and bladder cancer risk in humans. We conducted a large multicenter case–control study in Spain to explore the etiology of bladder cancer and evaluated the association between urine pH and cancer risk, alone and in combination with cigarette smoking.

Abbreviations: ABP, aminobiphenyl; CI, confidence interval; OR, odds ratio.

Materials and methods

Study design and data collection

Study methods have been described elsewhere (8,9). Briefly, we recruited 1219 incident transitional cell carcinoma cases (84% of 1453 contacted cases) and 1271 hospital controls (88% of 1442 controls) between June 1998 and June 2001 in 18 hospitals in the following regions in Spain: Barcelona, Valles/Bages, Asturias, Alicante and Tenerife. Cases were all male and female patients with newly diagnosed, transitional cell carcinoma of the bladder (International Classification of Diseases, Ninth Edition code 1880–1889) or carcinoma *in situ* (International Classification of Diseases, Ninth Edition code 2337) of the bladder, including uretric orafice and urachus, who were 21–80 years old at the time of diagnosis and resided in the catchment areas of the 18 participating hospitals. Diagnostic slides for each patient were reviewed by a panel of expert pathologists to confirm the diagnosis of bladder cancer and ensure uniformity of classification criteria based on the 1998 World Health Organization/International Society of Urological Pathology system (10), as well as the histologic subtype. Patients who had a previous diagnosis of cancer of the lower urinary tract (i.e. bladder, renal pelvis, ureters or urethra) were not eligible for study, and neither were patients with bladder tumors that were secondary to other malignancies. For each bladder cancer case, one control was selected and individually matched to the case on age (within 5 years), gender, race/ethnicity and hospital. Controls were selected from patients admitted to the same hospital around the same time as the cases for diseases/conditions unrelated to smoking (36% hernias, 12% other abdominal surgery, 12% hydrocele, 24% fractures, 6% other orthopedics, 4% circulatory diseases, 1% ophthalmology diseases, 2% dermatology diseases and 4% other diagnosis).

Because there were only six non-White subjects included in this study, our analyses are based exclusively on Whites. This study was approved by the National Cancer Institute Institutional Review Board as well as by the ethics committees of all participating hospitals.

All subjects were interviewed in the hospital using a computer-assisted personal interview. Before the interview, written informed consent to participate in the study was obtained from each subject. The questionnaire was designed to elicit detailed information on smoking habits, dietary factors, fluid intake, medical conditions, occupational and residential histories, family history of cancer and history of medication use (i.e. analgesics and non-steroidal anti-inflammatory drugs).

Study participants were trained to test their urine pH with dipsticks at home and record results into a diary. In total, 712 cases (59.2%) and 611 controls (48.1%) returned diaries with complete data on urinary pH, which they measured twice a day (first void in the morning and early in the evening) during four consecutive days 2 weeks after hospital discharge. We carried out a pilot study to develop a protocol to measure urine pH in the home as part of a case–control study. We found that essentially all individuals with urinary pH ≤6.0 for every first morning and evening void over a 4 day period maintained this pattern over a 7 day period; and that urine dipstick readings made by the kit used in this study were highly correlated with those obtained by a pH meter (Spearman $r = 0.95$; $P < 0.001$) (11). Subjects who returned a urine diary were similar to those who did not with regard to grams of fruits and vegetables intake, body mass index (BMI) and smoking status. The magnitude of the association between cigarette smoking and bladder cancer in the population with urinary pH was identical to that reported for the whole study (8,9). Subjects included in the urine pH analyses were on

average ~2 years younger ($P < 0.001$), less likely not to have finished primary school (39.2% versus 55.5%; $P < 0.001$), and more likely to be living with a spouse (81.8% versus 70.1%; $P < 0.001$), compared with subjects who were not included in the analyses. Subjects with all of their pH readings ≤6.0 (46.7% of cases, 38.9% of controls) were categorized as having a consistently acidic urine pH. As urine pH reflects several factors that can vary over time, we adopted this conservative definition to maximize the probability that such individuals would have had a long-term tendency to have acidic urine. The control group included subjects with a relatively wide variety of pathologies, and none of them was associated with having a consistently acidic urine pH. Among controls, sex, cigarette smoking status, alcohol consumption, enlarged prostate, nocturia, usual BMI, current BMI, grams of meat intake and grams of vegetables intake were not associated with having consistently acidic urine pH. Age, study region, finished studies, average thrihalometanes exposure and grams of fruit intake were associated with urine pH among controls (supplementary Table I is available at *Carcinogenesis* Online).

Statistical analysis

To estimate the effects of urinary pH on bladder cancer risk, we calculated odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditional logistic regression, with terms entered for exposure and potential confounding variables (i.e. age at interview, gender, geographic region, cigarettes per day and smoking duration). To test for linear trend, we computed the Wald statistic, treating the exposure variable as a continuous variable by entering the median value for each level of the categorical variable among control subjects. To test for interaction between two risk factors, we added a cross product term to the logistic model and conducted a likelihood ratio test.

Results

Table I shows risk of bladder cancer associated with urine pH. Having a urine pH that was consistently ≤6.0 was associated with a significant 1.5-fold increased risk of bladder cancer. When subjects with consistently acidic urine at pH ≤6.0 were further subdivided into those whose urinary pH was above pH 5.0 in one or more samples and subjects whose urine pH was always ≤5.0, a dose–response relationship was apparent (i.e. OR = 1.5 and 1.8, respectively, $P_{trend} = 0.001$, Table I). The dose–response trend was consistent among both men and women, although only statistically significant among men ($P_{trend} = 0.001$; 0.42 in women) (Table I).

Urine pH was not associated with bladder cancer among non-smokers (OR = 1.0; 95% CI: 0.6–1.8), weakly associated among former smokers (OR = 1.3; 95% CI: 0.9–1.9) and strongly associated among current smokers (OR = 2.1; 95% CI: 1.3–3.2) (Table II). Current smoking status was unrelated to having a consistently acidic urine pH among controls ($P = 0.59$). Overall risk estimates for cigarette intensity among current smokers were OR = 5.6 (95% CI: 3.3–9.5), 8.5 (5.3–13.8) and 9.1 (5.3–15.7) for smoking 1–19, 20–29 and 30+ cigarettes per day versus non-smokers, respectively. Figure 1 shows the combined effect of urine pH and smoking intensity for current smokers. Risk estimates for cigarette smoking intensity were consistently higher among those with acidic pH, with evidence of interaction among heavy current smokers who smoked ≥30 cigarettes/day (Pinteraction = 0.024). Further adjustment by smoking duration yielded the following risk estimates between smoking 1–19 cigarettes/day, 20–29 cigarettes/day and ≥30 cigarettes/day versus non-smokers bladder cancer and among current smokers: OR = 2.8 (95% CI: 0.8–9.2); 4.9 (1.6–15.0); 3.8 (1.2–12.1) among subjects without acidic pH and OR = 3.8 (95% CI: 1.2–11.7); 4.7 (1.6–13.9); 10.0 (2.8–36.0), among subjects with acidic pH ($P_{interaction}$ for difference in effects for ≥30 cigarettes/day = 0.016). Similarly, as with smoking intensity, risk estimates for all the categories of quartiles of duration smoking and quartiles of pack-years were higher among subjects with consistently acidic pH as compared with subjects without, although the interaction tests were not significant for the highest categories ($P_{interaction}$ for difference in effects for smoked ≥40 years = 0.28; ($P_{interaction}$ for difference in effects for smoked >35 pack-years = 0.17).

Adjustment for factors that could potentially influence urine pH, including diet composition (daily vegetable intake, fruit intake and/or combined vegetable/fruit intake), body surface area, trihalomethane ingestion, non-steroidal anti-inflammatory drugs and certain medications (12,13) (supplementary Table I is available at *Carcinogenesis* Online), as well as for education and marital status, had a minimal effect on point estimates for the associations reported (Table III). Also, the magnitude of the associations was the same in the populations with and without changes in BMI, residence, job and diet in the previous 5, 5–15 and >15 years to the interview (supplementary Table II is available at *Carcinogenesis* Online). Furthermore, urine pH did not change in any consistent pattern across stage (chi-square test P value = 0.30) or grade (chi-square test P value = 0.17), and the relationship between urine pH and risk of bladder cancer did not vary substantially by stage or grade, although evaluation of effects in subjects with advanced disease was limited by small numbers (Table IV). In addition, excluding controls with each major group of control diseases from the analyses did not affect results (data not shown).

Results were not altered by *NAT2* or *GSTM1* genotype or other more recently reported genetic variants that impact on risk of bladder cancer (14) and there was no strong evidence for multiplicative interactions between urine pH and these genetic markers (data not shown).

Table I. Risk of bladder cancer associated with urine pH

Urine pH	Number of cases	Number of controls	OR[a]	95% CI
All				
>6.0[b]	379	374	1.0	
≤6.0[c]	333	237	1.5	(1.2–1.9)
5.5–6.0[d]	284	209	1.5	(1.1–1.9)
≤5.0[e]	49	28	1.8	(1.1–3.0)[f]
Men				
>6.0	328	335	1.0	
≤6.0	297	212	1.5	(1.2–2.0)
5.5–6.0	253	188	1.5	(1.1–1.9)
≤5.0	44	24	1.9	(1.1–3.4)[f]
Women				
>6.0	51	39	1.0	
≤6.0	36	25	1.3	(0.6–2.7)
5.5–6.0	31	21	1.2	(0.6–2.7)
≤5.0	5	4	1.7	(0.4–7.6)

[a]OR and 95% CIs adjusted for age (five categories), study region, gender, cigarettes/day and duration of smoking.
[b]>6.0: at least one of eight urine pH values >6.0.
[c]≤6.0: all eight values ≤6.0.
[d]5.5–6.0: all values ≤6.0, with at least one value >5.0.
[e]≤5.0: all values ≤ 5.0.
[f]P value for the linear trend test = 0.001.

Table II. Risk of bladder cancer associated with urine pH, by cigarette smoking status

Urine pH	Number of cases	Number of controls	OR[a]	95% CI
Non-smokers				
>6.0[b]	67	114	1.0	
≤6.0[c]	39	67	1.0	(0.6–1.8)
Former smokers				
>6.0	168	176	1.0	
≤6.0	136	116	1.3	(0.9–1.9)
Current smokers				
>6.0	144	84	1.0	
≤6.0	158	54	2.1	(1.3–3.2)

[a]OR and 95% CIs adjusted for age (five categories), study region, gender, cigarettes/day and duration of smoking.
[b]>6.0: at least one of eight urine pH values >6.0.
[c]≤6.0: all eight values ≤6.0.

Discussion

Urine pH is determined primarily by a combination of body surface and dietary intake, where fruits and vegetables contribute to alkalinizing urine pH, whereas meat, fish and dairy products contribute to

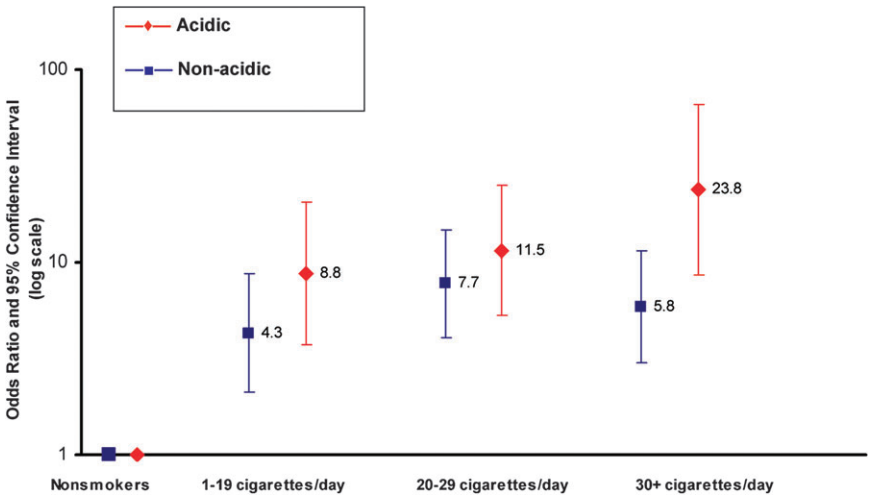

Fig. 1. Risk of bladder cancer by quartiles of cigarette smoking intensity among current smokers with and without consistently acidic urine pH. OR and 95% CI adjusted for gender, age (5 year categories) and hospital region. Consistently, acidic urine pH defined as having all eight pH readings ≤6.0 ($P_{interaction}$ for difference in effects for ≥30 cigarettes/day = 0.024).

Table III. Adjusted risk of bladder cancer associated with urine pH

Additional factor in the model[a]	Maximum urine pH[b]					
	≤6.0		5.5–6.0		5.0	
	OR	95% CI	OR	95% CI	OR	95% CI
No additional risk factor	1.5	(1.2–1.9)	1.5	(1.1–1.9)	1.8	(1.1–3.0)
Combined vegetables and fruits (g/day)	1.4	(1.1–1.8)	1.4	(1.1–1.8)	1.7	(1.0–2.9)
Vegetables (g/day)	1.4	(1.1–1.8)	1.4	(1.1–1.8)	1.7	(1.0–2.9)
Fruits (g/day)	1.4	(1.1–1.8)	1.4	(1.1–1.8)	1.7	(1.0–2.9)
BMI (usual)	1.4	(1.1–1.9)	1.4	(1.1–1.8)	1.8	(1.0–3.1)
BMI (current)	1.5	(1.1–1.9)	1.4	(1.1–1.8)	1.9	(1.1–3.3)
Medications[c]						
Low-ceiling diuretics, thiazides	1.5	(1.2–2.0)	1.5	(1.2–1.9)	1.9	(1.2–3.2)
Selective calcium channel blockers	1.5	(1.2–1.9)	1.5	(1.2–1.9)	1.9	(1.1–3.2)
Simvastatin	1.5	(1.2–1.9)	1.5	(1.2–1.9)	1.9	(1.1–3.1)
Nervous system	1.5	(1.2–1.9)	1.5	(1.1–1.9)	1.8	(1.1–3.0)
Cough suppressants, excluding combinations with expectorants	1.5	(1.2–1.9)	1.5	(1.1–1.9)	1.9	(1.1–3.1)
Non-steroidal anti-inflammatory drugs	1.4	(1.1–1.8)	1.3	(1.0–1.8)	1.7	(0.98–2.9)
Trihalomethane ingestion	1.5	(1.2–1.9)	1.5	(1.1–1.9)	1.7	(1.0–2.7)
Education	1.5	(1.2–1.9)	1.4	(1.1–1.9)	1.9	(1.1–3.3)
Marital status	1.4	(1.1–1.8)	1.3	(1.0–1.7)	1.9	(1.1–3.3)

[a]OR and 95% CIs also adjusted for age (five categories), study region, gender, cigarettes/day and duration of smoking.
[b]≤6.0: all eight values ≤6.0; 5.5–6.0: all values ≤6.0, with at least one value >5.0; ≤5.0: all values ≤5.0.
[c]All medications that in our study population were related to case/control status and to urine pH.

lowering urine pH (13). We observed a dose–response relationship in bladder cancer risk with increasing urinary acidity, with no association among non-smokers, a weak association among former smokers, a strong association among current smokers and with evidence of interaction between having consistently acidic urinary pH and heavy smoking. This is the first study to report an association between directly measured urinary pH and risk of bladder cancer in humans. Its major advantage is that we were able to identify subjects with consistently acidic urine, which we have shown previously requires taking measurements over several days (11). One prospective study that used data on nutrient intake and anthropometry to indirectly estimate each person's usual urine pH did not find an overall association between low urine pH and bladder cancer risk, although there was some evidence of increased risk among smokers with >45 pack-years (OR = 1.72, 95% CI: 0.96–3.10) (15). The association with consistently acidic urinary pH in our data produced similar results when restricting the analyses to the later group (OR = 1.67, 95% CI: 1.06–2.65).

In our study, total fluid intake was associated with a decrease in bladder cancer risk (16) and we have also reported a consistent inverse trend in bladder cancer risk with increasing number of voids per night among both men and women (17) which together with the findings presented in this report support the urogenous contact hypothesis as a probable mechanism for bladder carcinogenesis in humans. The lack of plateau in the dose–response curve between cigarette smoking intensity and bladder cancer risk among subjects with acidic pH but not among subjects without (Figure 1), suggests that urinary pH might contribute to carcinogen activation or influence the saturation of metabolic activation processes that might occur at high levels of tobacco exposure.

One concern is whether our urine pH classification at diagnosis time reflects the urine pH in the periods of life relevant for bladder carcinogenesis. If determinants of urine pH do not change over time, we can assume that urine pH will not change either. As the magnitude of the associations reported was the same in subgroups of the study population with and without changes over time in factors related to

Table IV. Risk of bladder cancer associated with urine pH by stage and by grade

Urine pH[a]	Stage[b]														
	Ta			T1			T2			T3			T4		
	n	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI
>6.0	250	1.0	Ref	39	1.0	Ref	52	1.0	Ref	12	1.0	Ref	11	1.0	Ref
≤6.0	232	1.6	(1.2–2.1)	35	1.5	(0.9–2.5)	28	0.9	(0.6–1.6)	16	2.5	(1.1–5.7)	7	1.2	(0.4–3.3)
5.5–6.0	201	1.6	(1.2–2.1)	27	1.3	(0.7–2.3)	26	1.0	(0.6–1.7)	13	2.4	(1.0–5.6)	6	1.2	(0.4–3.3)
≤5.0	31	1.6	(0.9–2.9)	8	2.7	(1.1–6.6)	2	0.5	(0.1–2.4)	3	3.5	(0.8–15)	1	1.4	(0.2–12)
Urine pH[a]	Grade[b]														
	LMP[c]			G1			G2			G3					
	n	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI			
>6.0	10	1.0	Ref	106	1.0	Ref	108	1.0	Ref	140	1.0	Ref			
≤6.0	16	2.4	(1.01–5.6)	99	1.6	(1.1–2.3)	107	1.8	(1.2–2.5)	96	1.2	(0.9–1.7)			
5.5–6.0	15	2.6	(1.1–6.2)	88	1.7	(1.2–2.4)	91	1.7	(1.2–2.5)	79	1.1	(0.8–1.6)			
≤5.0	1	1.1	(0.1–9.6)	11	1.3	(0.6–2.9)	16	1.9	(0.9–3.9)	17	1.7	(0.9–3.3)			

[a]Urine pH measured twice a day (early in the morning and early in the evening) during four consecutive days.
[b]OR adjusted for age (five categories), study region, gender, cigarettes/day and duration of smoking.
[c]LMP, low malignant potential.

urine pH such as BMI and diet, we think that it is unlikely that changes over time in urine pH influenced our conclusions to any substantial extent.

Since urinary pH was measured by cases after bladder cancer was diagnosed and treated, there is a concern that urinary pH may have been directly or indirectly influenced by the disease itself (18) or its treatment, resulting in a possible spurious association. We addressed this concern in several ways. First, we conducted a methodological study with 14 bladder cancer cases independent from those included in the case–control study to compare urinary pH measured for 4 days 1 week before hospital admission, and again 10 days and 3 weeks after hospital discharge in the same hospitals in two out of the five study areas where subjects for the case–control study were recruited. We found no differences in the median of the maximum pH ($P = 0.51$) and average pH ($P = 0.67$) of the two sets of measurements nor any consistent pattern. These results suggest that urine pH was not altered by either the disease or postsurgery status through the period of time that study subjects measured their urine pH at home following hospitalization. Secondly, we evaluated the impact of red blood cells in urine on the pH in an experimental study with urine samples from seven healthy volunteers. Five samples had acidic pH, one had neutral pH and one had alkaline pH. We added up to 10 μl of red blood cells in 10 ml of urine and measured urine pH every 2–3 h until 12 h after the first measurement (a period of time longer than the average time that urine might spend in the human bladder). We observed no urine pH changes in any of the urine samples. Thirdly, urine pH was not associated with grade or stage of the disease, indicating that neither disease progression nor its treatment were likely to influence urine pH (Table IV). Finally, excluding each major group of control diseases from the control group did not affect results (data not shown).

In conclusion, we report that urine pH may modify the impact of tobacco use on risk of bladder cancer in a way that is consistent with experimental data showing that acidic urine can result in cleavage of acid-labile glucuronides of carcinogenic aromatic amines (1). Our findings, which need to be replicated, provide new insights into the pathogenesis of bladder cancer and suggest that non-genetic host factors may be important sources of individual susceptibility for this tumor.

Supplementary material

Supplementary Tables I and II can be found at http://carcin.oxfordjournals.org/

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