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ORIGINAL ARTICLE

Risk of bladder cancer in male Japanese workers exposed to *ortho*-toluidine and other aromatic amines

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Abstract

Purpose Nine bladder cancer (BCa) cases were reported among aromatic amine-exposed male workers at a factory manufacturing organic dye/pigment intermediates in Japan. We aimed to evaluate the characteristics of aromatic amine-exposed workers by cross-sectional observation, and the risk of BCa by assessing the standardized incidence ratio (SIR).

Methods In the cross-sectional study, our subjects were: 9 BCa patients, 36 aromatic amine-exposed non-patients, and 79 non-exposed workers from 3 factories. We evaluated the subjects' medical history, urinalysis, qualitative determination of nuclear matrix protein 22, and urinary cytology. For SIR assessment, 98 aromatic amine-exposed workers from 1 factory were included, and the Japanese general male population was used as a referent population. Since no direct aromatic amine-exposure data were available, we calculated surrogate exposure levels using information on job sites, exposure potency, and duration.

Results Coexistent aromatic amines were *ortho*-toluidine (OT), aniline, *para*-toluidine, *ortho*-anisidine, 2,4-xylidine, and *ortho*-chloroaniline. The prevalence rates of cystitis and bladder lesion-related symptoms in both BCa patients and aromatic amine-exposed non-patient workers were significantly higher than those of non-exposed workers. Overall, the SIR for BCa in OT-exposed workers was 56.8 (95% CI 27.7–104.3) and apparent dose–response relationships were revealed between the SIR and the surrogate exposure level in the 0–10-year lagged analyses. Overall, SIRs in other aromatic amine-exposed workers were also significantly high but no or unclear dose–response relationships were observed.

Conclusions We conclude that OT may be responsible for the increased risk of BCa. Regular monitoring of bladder lesion-related symptoms is essential for the early identification of BCa.

Keywords *ortho*-Toluidine · Bladder cancer · SIR · Cross-sectional study · 2,4-Xylidine · Aniline

Abbreviations

AN	Aniline
BCa	Bladder cancer
MX	2,4-Xylidine
OA	*ortho*-Anisidine
OCA	*ortho*-Chloroaniline
OT	*ortho*-Toluidine
PT	*para*-Toluidine
SIR	Standardized incidence ratio

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Introduction

ortho-Toluidine (OT) is a known human carcinogen. It is absorbed through several routes, including inhalation, ingestion, and skin contact. OT was listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) in 2012 (IARC 2020). However, the products made from OT are still used worldwide (National Toxicology Program 2014).

Several cohort studies have been conducted among workers exposed to OT and other aromatic amines. Ward et al. (1991) reported SIR 6.48 of BCa in 708 US workers exposed to OT and aniline, and SIR 3.60 in all 1749 workers at a rubber additive production plant. Increased risk of BCa was strongly associated with increased duration of employment. Using the revised exposure categories, Carreón et al. (2010) rereported SIR 5.84 of BCa in 962 workers exposed to OT and aniline. Sorahan (2008) reported increased risks between OT exposure and BCa in 611 workers exposed to one or more of four chemicals: 2-mercaptobenzothiazole, aniline, phenyl-β-naphthylamine and OT. In occupational fields, it is often difficult to assess the excess risk of OT alone and BCa due to the mixed exposure process of aromatic amines and other chemicals.

In Japan, five male workers, including a retiree, of a small-sized factory A of a company that produces organic dyes and pigment intermediates, were diagnosed with bladder cancer (BCa) between 2014 and 2015 following gross hematuria. Subsequently, the company started health checkups two to four times a year, intensively focusing on the detection of urinary tract cancer. An additional four male workers at the same factory and one male worker at another small-sized factory B at the same company were also diagnosed with BCa in 2016 and 2017. All these individuals were exposed primarily to OT and partially to aniline (AN), 2,4-xylidine (MX), *para*-toluidine (PT), *ortho*-anisidine (OA) and/or *ortho*-chloroaniline (OCA). We have previously reported these ten cases (Nakano et al. 2018).

This report presents the results of two analyses. First, this is a cross-sectional analysis comparing characteristics, past clinical history, current/past symptoms of urinary tract diseases, and results of urinalyses of the non-exposed and aromatic amine-exposed groups, including the nine patients of BCa from factory A. The non-exposed subjects were selected from factories A and B, and another factory C of the same company. The non-exposed subjects were not exposed to any of OT, AN, MX, PT, OA and OCA. Second, this is an analysis of the risk for BCa due to exposure to OT and other aromatic amines.

Methods

This study was approved by the Ethics Committee of the School of Medicine of Keio University (approval number: 20160172). Written consent was obtained from all subjects.

Cross-sectional study

Study population

Factory A had a total 120 employees with records since 1988, while factories B and C at the same company had 420 employees. Subjects exposed to OT or any other aromatic amines (PT, OA, AN, MX or OCA) were defined as exposed. Exposure cutoff levels were chosen as more than 1 day of exposure per month. An exposed subject at factory B was diagnosed with BCa. However, since the detailed exposure records of factories B and C were not available, valid exposure assessments could not be performed. In factories B and C, the amounts of aromatic amine consumption were smaller and the exposure levels were lower than at factory A. Therefore, we decided to exclude aromatic amine-exposed workers in factories B and C ($n = 133$) from the cross-sectional analyses to properly assess the effects of aromatic amine exposure on workers at factory A with the BCa epidemic. We also excluded exposed retired subjects with unknown exposure status ($n = 86$).

Finally, the company's files had job history records for 321 subjects (205 non-exposed; 116 exposed). Of these, 119 non-exposed and 70 exposed subjects were eliminated due to missing information: 5 unknown birth dates, 10 deaths and 104 addresses unknown or no response in the non-exposed group; 4 deaths, 9 unknown detailed exposure status, 4 unknown addresses, and 53 declined health checkups or no response in the exposed group. 46 exposed subjects from factory A were included in the study based on the higher incidence of BCa at that location. On the other hand, 86 subjects not exposed to these aromatic amines were from factories A, B and C as non-exposed controls. A cross-sectional study was conducted using detailed information regarding the job history and health checkups of these workers that were provided by the company and conducted by the researchers. For three workers who had more than 5-year exposure and who did not participate in health checkup conducted by the researchers, we got their responses for an only self-administered questionnaire. We also confirmed that these three exposed workers had not been diagnosed with BCa. The causes of the deaths of employees before this study

in the non-exposed group included lung cancer (1), pharyngeal cancer (1), pancreatic cancer (1), brain contusion (1), pneumonitis (1), and unknown (5). On the other hand, the causes of death in the exposed group were pharyngeal cancer (1), suicides (2) and unknown (1).

Health checkup

We assessed the past/current medical symptoms and history of the participants using a questionnaire followed with physician interviews. Urinalysis was performed to assess the expression of nuclear matrix protein 22 (NMP-22), a marker of BCa, and for cytology. The cutoff value for NMP-22 was 12.0 U/mL (normal range, < 12.0) (Akaza et al. 1997).

Standardized incidence ratio (SIR)

Study population of factory A

While confirming lost cases due to retirement in an analysis of risk for BCa, the study was expanded to include workers hired after 1988 at factory A. The subjects for this study component were employees ($n = 116$) who were exposed to any of the six aromatic amines in factory A since 1988, and had available medical records. Following the evidence of BCa in December 2017, detailed information on their vital status and past clinical history was obtained from the company. Six women with almost no exposure to aromatic amines and no BCa, and 12 workers with no detailed information regarding the status (*n*, 9) or duration (*n*, 3) of their exposure were excluded from the study. Finally, 98 out of 116 workers (84.5%) from factory A were included in the SIR analysis.

Using the cancer statistical data (Hori et al. 2015), we calculated the mean age-specific, 5-year incidence rate of BCa in men from 2003 to 2015. The observation period was calculated from the day of the initial exposure to any of the six aromatic amines to the time of examination for the cross-sectional study. The end point of the observation period was February 10, 2017. Time was censored as follows: (a) December 1, 2016 for subjects confirmed their health status by the company without our examination, (b) the retirement day (by company records) for subjects not confirmed their health status, (c) the day of diagnosis for patients with BCa, and (d) the day of death, or the first day of the month or year of death for subjects who had died.

In the analyses for SIR among OT and other aromatic amine-exposed workers, the starting time of observation period was considered as the day of the initial exposure to each aromatic amine.

Time lag calculation

To account for a possible latent duration between exposure and its consequences, cumulative exposure durations were calculated using a range of different lag times. Since the lag time to the incidence of BCa in the aromatic amine-exposed workers was unclear, we calculated exposure doses by assuming lag times of 0, 5, 10, and 15 years. For example, assuming the lag time of 5 years, the exposure period was from the start of observation until 5 years before the end of observation. When aromatic amines were involved in the incidence of BCa, we considered that the latency period could be estimated by setting some lag times and examining the relationship between the SIR and estimated exposure in the period excluding the lag time from the original observation period.

Work description

The OT-related processes included (1) preparation and reaction (acetoacetylation) by mixing OT and diketene in an organic solvent, (2) filtering and rinsing the crude product with an organic solvent, (3) drying the rinsed wet product, and packing it (powder), and (4) distilling the waste organic solvent for reuse. The rinsing liquid containing small amounts of OT was distilled in process (4) and reused. The percentage of OT in processes 1, 4, 2, and 3 were 99%, about 15%, 0.1–1.5% and < 0.1%, respectively. Since processes 1 and 4 are carried out in closed tanks, they are considered to involve a relatively lower exposure to OT (through the skin and/or respiratory tract). However, process 2 which is carried out in open tanks and process 3, which requires manually transferring the product into containers, involve relatively higher exposures to OT (Nakano et al. 2018).

Surrogate exposure level estimation

Since no previous quantitative OT exposure data were available, we estimated the surrogate exposure levels of OT and other aromatic amines. Surrogate exposure level, defined as the total job-weight-month for each process for each job-year, was calculated based on the company records, interviews with long-term employees, and extensive data on job-weight-month for each of the four processes and job-year of annual exposure to OT and other amines. Job-weight-month was allocated as follows: 0 (none), 1 (l–2 days per month), 5 (3–9 days per month), or 10 (more than 10 days per month). The details of the calculation have been described previously (Nakano et al. 2018).

Confirmation of the diagnosis bladder cancer

Diagnosis of all BCa cases was confirmed by pathological examination.

Smoking adjustment

Smoking is one of the most important risk factors for BCa (Freedman et al. 2011). Therefore, it is possible to misestimate the risk of BCa by exposure to aromatic amines when smoking status differs between male workers and Japanese males included in the SIR calculation. Following the study by Carreón et al. (2014), we, therefore, used national data to estimate the bias factor of smoking and to investigate the effect of differences in smoking status between the two groups on the SIR calculation.

Of the 42 male workers exposed to aromatic amines for whom we had smoking histories, 12 were non-smokers (28.6%), 13 were former smokers (31.0%), and 17 were current smokers (40.5%) when smoking status was checked at the median observation period of each worker. When the smoking status of Japanese males was checked in 2003, among 4204 research subjects, 1358 were non-smokers (32.3%), 879 former smokers (20.9%), and 1967 current smokers (46.8%) (Ministry of Health, Labor and Welfare 2003). The hazard ratios (HR) for BCa among Japanese males were reported to be 1.32 (95% confidence interval, CI 0.80–2.16) among former smokers, 1.69 (95% CI 1.09–2.63) among current smokers, and 1 among non-smokers. (Kurahashi et al. 2009).

The bias factor was calculated as follows:

$$\text{bias factor} = \frac{P_{NW} \times 1 + P_{FW} \times HR_F + P_{CW} \times HR_C}{P_{NJ} \times 1 + P_{FJ} \times HR_F + P_{CJ} \times HR_C},$$

where P_{NW} is the proportion of non-smokers among the male workers, P_{FW} is the proportion of former smokers among the male workers, P_{CW} is the proportion of current smokers among the male workers, P_{NJ} is the proportion of non-smokers among Japanese males, P_{FJ} is the proportion of former smokers among Japanese males, P_{CJ} is the proportion of current smokers among Japanese males, HR_F is the hazard ratio for BCa of former smokers, and HR_C is the hazard ratio for BCa of current smokers.

Statistics

Cross-sectional study

The participants ($n = 132$) in our study were divided into 2 groups; 86 non-exposed and 46 aromatic amine-exposed workers. The subjects who did not give informed consent ($n = 1$) or those who were absent from our examination ($n = 7$) were excluded from the analysis. In addition, to clarify the characteristics of the patients with BCa, the exposed group was further divided into two groups of BCa patients ($n = 9$) and non-patients ($n = 36$). The Kruskal–Wallis test was used to compare the continuous variables between the non-exposed, BCa patients, and non-patients groups. The χ^2 test or Fisher's exact test was used to compare proportions and prevalence. Since there were the significant differences in the prevalence of diseases between the non-exposed group, non-patient group and BCa patient group, the relationship between the surrogate exposure levels of OT and cystitis, high blood pressure, and diabetes mellitus was analyzed, using a logistic regression model and adjusting for age.

SIR study

Correlation coefficients between cumulative surrogate exposure to aromatic amines and observed person-years were analyzed using Pearson's correlation coefficient. The trends in the categories of surrogate exposure levels were determined using the Kendall rank correlation coefficient.

Smoking adjustment

The bias factor of smoking was estimated by Bayesian inference using Markov Chain Monte Carlo methods (MCMC) with the RStan package version 2.21.2.

For both, the cross-sectional study and the SIR study, statistical significance was assessed by the two-tailed analysis, and $p < 0.05$ was considered significant. All statistical analyses were performed using SPSS v. 24 (IBM Corporation, Armonk, NY, USA), OpenEpi v. 30.1 (Dean et al. 2013) and EZR v. 1.41 (Kanda 2013).

Results

Figure 1 shows the flow of subjects whose records since 1988 were available. Nine cases of BCa were diagnosed in factory A between 2014 and 2017.

Cross-sectional study

Characteristics of BCa patients

Table 1 summarizes the characteristics of the non-exposed and exposed subjects (BCa patients and non-patients). All nine BCa patients were men and were alive in 2017. The mean age of the subjects was 57.6 years, and smoking status was one never, six ex-, and two current smokers. Retired workers accounted for 33.3% of the study population. The

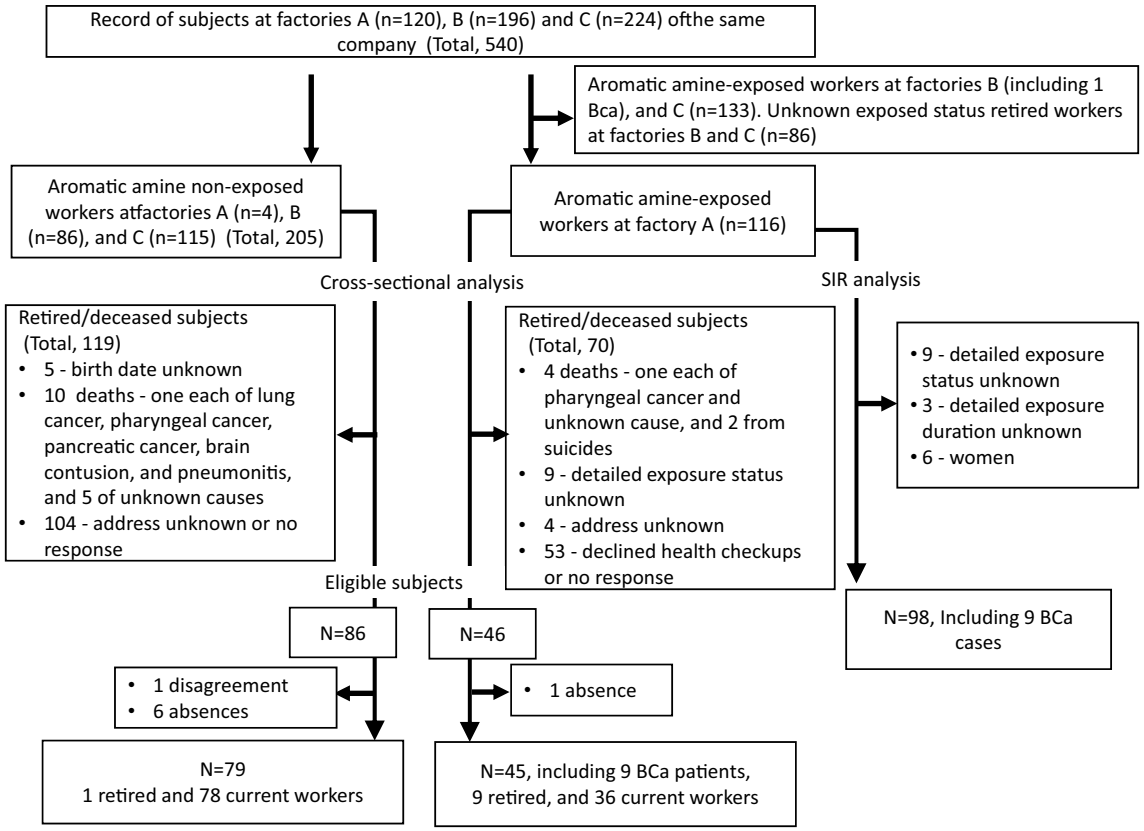

Fig. 1 Company record of subjects in the company job history files since 1988

mean duration of exposure to OT was 19.6 years. While the mean age of the BCa patients was higher compared to that of the non-patients, there was no difference in their employment duration. All nine BCa patients had been exposed to OT, MX, AN, OA, and OCA. However, OA and OCA were disproportionately correlated with BCa. The time of initial exposure ranged from 1988 to 1997. All the BCa patients had worked for more than 5 years, and the longest employment duration was 20–30 years. While eight of the nine patients were engaged in process 2; filtering and rinsing the crude product with an organic solvent, all of them were engaged in process 3; drying the rinsed wet product and packing it (powder). Notably, four very different age distributions (40–49, 50–59, 60–69 and 70–79) were shown in BCa patients. This result showed the increased occurrence of BCa with latency.

Clinical history, symptoms of urinary tract diseases and results of urinalyses

Table 2 summarizes the past clinical history, current/past symptoms of urinary tract diseases and results of urinalyses of all the subjects. Of the BCa patients, each had a clinical history of four cystitis, four high blood pressure, and two diabetes mellitus. While the surrogate exposure levels of OT showed no significant relationship with high blood pressure ($p = 0.61$) and diabetes mellitus ($p = 0.90$), it was related to the incidence of cystitis ($p = 0.01$). While one patient in the non-exposed group had a previous history of breast cancer, another exposed non-patient had a gastric submucosal tumor. The incidence of atopic dermatitis was comparable among the BCa patients and the non-exposed group.

A study of past symptoms of urinary tract disease showed that gross hematuria was especially higher among the BCa patients compared to the other groups. Compared to the non-exposed group, the exposed non-patients had a higher incidence of gross hematuria (36.1 vs. 2.5%), urination pain (34.3 vs. 3.8%), feeling of residual urine (33.3 vs. 12.7%) and frequent urination (38.9 vs. 19.0%).

Results of the urinalysis revealed that 33.3% of the BCa patients were positive for NMP-22, which was higher than that in the other groups. BCa patients had treatment of transurethral resection surgery or cystectomy after diagnosis; however, one-third of all showed high urinary NMP-22 level (normal range, < 12.0).

Table 1 Characteristics of subjects exposed and non-exposed to OT and other aromatic amines

		Non-exposed	Exposed	
			Non-patients	BCa patients
		(*n* = 79)	(*n* = 36)	(*n* = 9)
Male, *n* (%)		65 (82.3)	34 (94.4)	9 (100)
Age, years (SD)		35.8 (12.6)	45.9 (10.7)	57.6 (9.1)**
Category, *n* (%)				
	19–29	32 (40.5)	3 (8.3)	0 (0.0)
	30–39	19 (24.1)	4 (11.1)	0 (0.0)
	40–49	17 (21.5)	17 (47.2)	2 (22.2)
	50–59	5 (6.3)	10 (27.8)	3 (33.3)
	60–69	6 (7.6)	2 (5.6)	3 (33.3)
	70–79	0 (0.0)	0 (0.0)	1 (11.1)
Smoking, *n* (%)				
	Never	35 (44.3)	11 (30.6)	1 (11.1)
	Ex	14 (17.7)	10 (27.8)	6 (66.7)
	Currently	30 (38.0)	15 (41.7)	2 (22.2)
Working status	Retired	1 (1.3)	6 (16.7)	3 (33.3)
Employment duration, years (SD)		12.1 (10.8)	17.5 (9.2)	19.6 (7.9)**
	< 1	10 (12.7)	0 (0.0)	0 (0.0)
	1–< 5	18 (22.8)	3 (8.3)	0 (0.0)
	5–< 10	17 (21.5)	3 (8.3)	2 (22.2)
	10–< 20	15 (19.0)	18 (50.0)	2 (22.2)
	20–< 30	14 (17.7)	10 (27.8)	5 (55.6)
	30–< 50	4 (5.1)	2 (5.6)	0 (0.0)
	Missing	1 (1.3)	0 (0.0)	0 (0.0)
Year of initial exposure to OT, *n* (%)				
	1980–1987	–	1 (2.8)	0 (0.0)
	1988–1995	–	12 (33.3)	6 (66.7)
	1996–2003	–	13 (36.1)	3 (33.3)
	2004–2015	–	7 (19.4)	0 (0.0)
	Unexposed	79 (100)	3 (8.3)	0 (0.0)
Exposure to aromatic amines, *n* (%)				
	OT	–	33 (91.7)	9 (100)
	MX	–	33 (91.7)	9 (100)
	AN	–	34 (94.4)	9 (100)
	OA	–	31 (86.1)	9 (100)
	OCA	–	31 (86.1)	9 (100)
	PT	–	16 (44.4)	2 (22.2)

**$P < 0.01$ by Kruskal–Wallis test among three groups

Analyses of SIR

Table 3 shows the correlation between the cumulative surrogate exposure to aromatic amines and observed person-years. Although there was a strong correlation in PT, the number of workers exposed to PT was small, and overall exposure was not necessarily correlated with person-years of observation.

Figure 2 compares the mean calendar year age-specific incidence rates of BCa (including carcinoma in situ; CIS) from 2003 to 2015 in the general Japanese male population and the number of BCa patients (including two CIS) in this study. While the mean incidence rate in general Japanese male population had been increasing in the over 60 years age group, BCa patients in this study showed the increased occurrence of BCa in the older than 40 years age group.

Figure 3 compares incidence rate of BCa (including CIS) by age group in the 2003–2015 general Japanese male population and the BCa patients (including two CIS) in this study. The figure shows that incidence rate of BCa in the aromatic

Table 2 Clinical history, symptoms of urinary tract diseases and results of urinalyses

	Non-exposed	Exposed	
		Non-patients	BCa patients
	(*n* = 79)	(*n* = 36)	(*n* = 9)
Past clinical history			
Urinary tract infection	0/79 (0.0)	1/34 (2.9)	0/9 (0.0)
Cystitis	4/79 (5.1)	12/36 (33.3)**	4/9 (44.4)**
Ureteral stones	1/79 (1.3)	6/36 (16.7)	2/9 (22.2)**
Heart disease	4/79 (5.1)	2/36 (5.6)	2/9 (22.2)
High blood pressure	7/79 (8.9)	10/36 (27.8)*	4/9 (44.4)**
Diabetes mellitus	1/79 (1.3)	2/36 (5.6)	2/9 (22.2)**
Kidney cancer	0/79 (0.0)	0/36 (0.0)	0/9 (0.0)
Other cancer	1/79 (1.3)	1/36 (2.8)	0/9 (0.0)
Methemoglobinemia	0/78 (0.0)	0/36 (0.0)	0/9 (0.0)
Atopic dermatitis	9/79 (11.4)	3/35 (8.6)	1/9 (11.1)
Skin rash/eczema	16/78 (20.5)	4/35 (11.4)	2/9 (22.2)
Symptoms of urinary tract disease at the time of the survey			
Gross hematuria	0/79 (0.0)	0/36 (0.0)	1/9 (11.1)**
Urination pain	0/79 (0.0)	0/36 (0.0)	1/9 (11.1)**
Feeling of residual urine	7/79 (8.9)	2/36 (5.6)	2/9 (22.2)
Frequent urination	8/79 (10.1)	7/36 (19.4)	4/9 (44.4)*
Past symptoms of urinary tract disease			
Gross hematuria	2/79 (2.5)	13/36 (36.1)**	7/9 (77.8)**
Urination pain	3/79 (3.8)	12/35 (34.3)**	4/9 (44.4)**
Feeling of residual urine	10/79 (12.7)	12/36 (33.3)**	3/9 (33.3)*
Frequent urination	15/79 (19.0)	14/36 (38.9)*	4/9 (44.4)*
Urine analysis findings[a]			
Proteinuria (≧1 +)	1/78 (1.3)	0/33 (0.0)	1/9 (11.1)
RBC (≧5/HPF)	0/78 (0.0)	0/33 (0.0)	1/9 (11.1)**
WBC (≧10/HPF)	1/78 (1.3)	0/33 (0.0)	0/9 (0.0)
NMP-22 (> 12 U/mL)	0/78 (0.0)	0/33 (0.0)	3/9 (33.3)**
Urine cytology			
Class 1&2	78/78 (100)	32/33 (97.0)	8/9 (88.9)
Class 3/3A	0/78 (0.0)	1/33 (3.0)	1/9 (11.1)

*, **$P < 0.05$, 0.01 compared to Non-exposed by $\chi 2$ test or Fisher's exact test

[a]*n* 78, 33 and 9 in non-exposed, non-patients and BCa patients, respectively

amine-exposed male workers was substantially higher in all age groups above 40 years of age. Note that because there were no cases of BCa in the workers under 39 years of age, and we did not include this group.

Table 4 shows the assessed risk of BCa in Japanese male workers exposed mainly to OT and other aromatic amines. Among 98 workers at factory A, who accounted for 1880.9 person-years-at-risk, 9 cases of BCa were reported. The expected number of BCa cases calculated for the Japanese male general population was 0.159, and the SIR for BCa in aromatic amine-exposed workers ($n = 98$) was 56.6 (9/0.159) (95% CI 27.6–103.9). The SIR for BCa in OT-exposed workers ($n = 94$) was 56.8 (9/0.158) (95% CI 27.7–104.3). To account for a possible latent duration between exposure and its consequences, cumulative exposure durations were calculated using a range of different lag times (0, 5, 10, and 15 years). For the unlagged, 5-year lagged, and 10-year lagged analyses, a significant relationship was seen between the surrogate exposure levels of OT and SIR ($p = 0.02$, 0.02 and 0.04, respectively). In addition, the surrogate exposure levels of OA, PT, and OCA were smaller than those of OT, MX, and AN. The surrogate exposure level of MX in MX-exposed workers ($n = 92$) showed no significant relationship with SIR. On the other hand, the surrogate exposure level of AN in AN-exposed workers ($n = 95$) showed a significant relationship with SIR only in the 5-year lagged analysis ($p = 0.02$).

Smoking adjustment

The posterior median for the bias factor of smoking was estimated to be 1.00 (95% Bayesian credible interval: 0.89–1.11). We did not adjust SIR because this result indicated that the difference in smoking status between the workers exposed to aromatic amines and Japanese males in general was not sufficient to affect SIR.

Discussion

Cross-sectional study

Our findings highlight the early signs of BCa, such as cystitis and gross hematuria in workers exposed to OT and other aromatic amines. Since one-third of the exposed non-patients had cystitis and urinary tract symptoms (gross hematuria, urination pain, feeling of residual urine and frequent urination), it is critical to monitor the health of workers with these symptoms to detect BCa in its early stages.

The National Institute of Occupational Safety and Health in Japan has reported a correlation between OT contamination on the gloves and its detection in urine (Japan National Institute of Occupational Safety and Health 2016). Skin is an important route of exposure to OT. Processes 2 and 3 involve exposure to OT levels < 1.5 and < 0.1%, respectively, as in eight out of all patients and all patients. Hence, processes should aim to control and reduce dermal exposure of workers to OT and other aromatic amines. Studies in rats have shown that exposure to acetoacet-O-toluidide (AAOT), a product of OT acetoacetylation, and its subsequent metabolism leads to OT in the urine (Okuno et al. 2019). Good

Table 3 Correlation between cumulative surrogate exposure and observed person-years

	All cases			Non-BCa cases			BCa cases		
	N	PCC	95% CI	*N*	PCC	95% CI	*N*	PCC	95% CI
All workers	98	0.24	0.05–0.42	89	0.21	0.00–0.40	9	0.17	− 0.55–0.75
ortho-Toluidine (OT) exposure	94	0.2	0.00–0.39	85	0.16	− 0.06–0.36	9	0.24	− 0.50–0.78
2,4-Xylidine (MX) exposure	92	0.14	− 0.06–0.34	83	0.12	− 0.10–0.33	9	0	− 0.67–0.66
Aniline (AN) exposure	95	0.22	0.01–0.40	86	0.2	− 0.01–0.40	9	− 0.01	− 0.67–0.66
ortho-Anisidine (OA) exposure	64	0.22	− 0.03–0.44	55	0.19	− 0.08–0.43	9	0.31	− 0.44–0.81
ortho-Chloroaniline (OCA) exposure	78	0.27	0.05–0.46	69	0.27	0.03–0.48	9	0.22	− 0.52–0.77
para-Toluidine (PT) exposure	18	0.61	0.20–0.84	16	0.62	0.19–0.86	2	n.a	

BCa Bladder cancer, *PCC* Pearson's Correlation Coefficient, *CI* Confidence interval

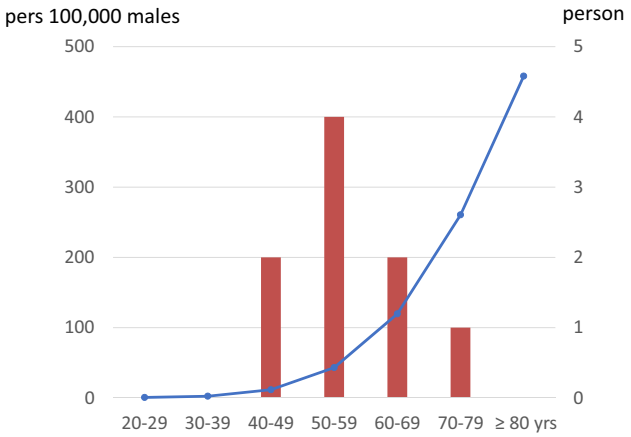

Fig. 2 Number of bladder cancer patients in this study (bar) and the average rates of annual bladder cancer incidence in the Japanese male population between 2003 and 2015 (polygonal line) by age categories

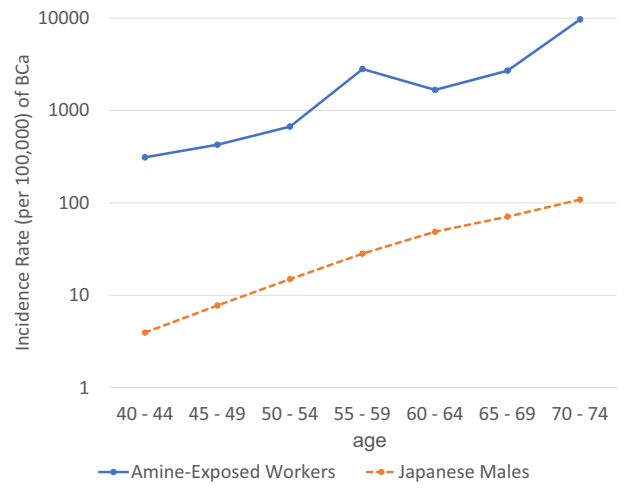

Fig. 3 Incidence rate by age group of BCa

hygiene practices for all exposure routes (inhalation, ingestion, and skin contact) should, therefore, be emphasized.

BCa patients older than 40 years showed the increased occurrence of BCa in this study. At factory A, though the BCa cases were detected between 2014 and 2017, the initial exposure to OT was between 1988 and 1997. The use of OT in the processes had been increasing from 1989 at factory A, which can explain the subsequent increase in the number of BCa cases at that location. The disease latency period following OT exposure is reported to be 20 years (Nakano et al. 2018), and all the BCa cases evaluated in this study involved exposure duration of more than 7 years. The mean duration of exposure to OT and the disease latency period in our study were comparable to those in another cohort study (Carreón et al. 2014). In a Japanese study of 126 cases of BCa from 1949 to 1972, with exposure to aromatic amines (benzidine, 1-naphthylamine or 2-naphthylamine), the mean disease latency period of BCa was 17 years (range 1–35) (Ishizu and Hashida 2007). Therefore, a latency period of 20 years is important characteristics of BCa. Based on the latency, it might indicate the likelihood for increased risk for BCa with time.

BCa patients had treatment of transurethral resection surgery or cystectomy after diagnosis; however, one-third of all showed high urinary NMP-22 level. Urinary NMP-22 is a useful biomarker for the monitoring of patients with non-muscle invasive BCa, and is thought to be released from the nuclei of tumor cells during apoptosis. Its expression is significantly higher in BCa tissue than in normal bladder epithelium (Akaza et al. 1997; Soloway et al. 1996). In addition to age and urinary cytology, urinary NMP-22 levels were a significant predictor of transitional cell carcinoma recurrence (Shariat et al. 2005). Despite treatment, the recurrence for BCa with 5 years was 47%, with half recurring within 1 year (Kikuchi et al. 2009). BCa patients with high urinary NMP-22 level in this study might be a sign of recurrence, and more careful observation for recurrence is needed.

Table 4 Risk of BCa in Japanese male workers mainly exposed to OT and other aromatic amines

	Surrogate exposure level	Person-year	Observed number	Expected number	SIR	95% CI
All workers ($n=98$)		1880.9	9	0.159	56.6	27.6–103.9
ortho-Toluidine (OT) exposure ($n=94$)	All	1827.0	9	0.158	56.8	27.7–104.3
Unlagged	0 < – < 50	995.2	0	0.085	0	n/a
	50– < 100	352.4	0	0.017	0	n/a
	100– < 200	297.9	2	0.032	62.0	10.4–204.8
	200– < 300	106.7	2	0.010	192.4	32.3–635.6
	300 -	74.8	5	0.014	363.3	133.1–805.2
					0.95 ($P=0.02$)	
5-year lagged	0	447.0	0	0.009	0	n/a
	0 < – < 50	797.3	0	0.084	0	n/a
	50– < 100	265.0	0	0.017	0	n/a
	100– < 200	217.1	4	0.032	125.2	39.8–301.9
	200– < 300	62.7	1	0.008	125.2	6.3–617.7
	300 -	38.0	4	0.009	447.4	142.2–1079.0
					0.89 ($P=0.02$)	
10-year lagged	0	872.6	0	0.026	0	n/a
	0 < – < 50	584.2	0	0.077	0	n/a
	50– < 100	188.9	0	0.018	0	n/a
	100– < 200	132.6	5	0.028	177.5	65.0–393.4
	200– < 300	33.1	3	0.006	471.4	119.9–1283.0
	300 -	15.7	1	0.003	296.7	14.9–1463.0
					0.75 ($P=0.04$)	
15-year lagged	0	1255.7	0	0.052	0	n/a
	0 < – < 50	380.9	0	0.067	0	n/a
	50– < 100	109.5	4	0.016	243.8	77.5–588.0
	100– < 200	62.7	3	0.019	159.6	40.6–434.3
	200– < 300	8.6	2	0.002	1313.0	220.1–4338.0
	300 -	9.6	0	0.002	0	n/a
					0.3 ($P=0.42$)	
2,4-Xylidine (MX) exposure ($n=92$)	All	1792.4	9	0.156	57.7	28.2–105.9
Unlagged	0 < – < 50	1021.8	0	0.086	0	n/a
	50– < 100	320.9	1	0.019	51.4	2.6–253.5
	100– < 200	319.2	4	0.034	119.1	37.8–287.2
	200– < 300	82.8	3	0.008	355.9	90.5–968.5
	300 -	47.8	1	0.008	119.1	6.0–587.4
					0.8 ($P=0.08$)	
5-year lagged	0	437.0	0	0.009	0	n/a
	0 < – < 50	829.6	0	0.085	0	n/a
	50– < 100	239.8	1	0.020	49.0	2.5–241.9
	100– < 200	217.1	6	0.031	194.0	78.6–403.6
	200– < 300	43.5	2	0.006	326.4	54.7–1078.0
	300 -	25.4	0	0.005	0	n/a
					0.45 ($P=0.23$)	
Aniline (AN) exposure ($n=95$)	All	1836.7	9	0.157	57.5	28.0–105.5

Table 4 (continued)

	Surrogate exposure level	Person-year	Observed number	Expected number	SIR	95% CI
Unlagged	0 < – < 50	940.1	0	0.081	0	n/a
	50– < 100	333.3	1	0.017	60.0	3.0–296.0
	100– < 200	355.6	5	0.036	138.6	50.8–307.2
	200– < 300	116.5	2	0.012	161.5	27.1–533.7
	300 -	91.2	1	0.011	94.3	4.7–465.2
					0.6 ($P=0.23$)	
5-year lagged	0	447.0	0	0.009	0	n/a
	0 < – < 50	746.2	0	0.079	0	n/a
	50– < 100	265.5	2	0.018	111.6	18.7–368.6
	100– < 200	260.5	4	0.034	118.4	37.6–285.7
	200– < 300	75.2	2	0.011	183.7	30.8–607.0
	300 -	42.4	1	0.006	165.0	8.3–813.6
					0.83 ($P=0.02$)	
ortho-Anisidine (OA) exposure ($n=64$)	All	1022.4	9	0.106	84.7	41.3–155.5
Unlagged	0 < – < 50	775.9	3	0.082	36.6	9.3–99.6
	50– < 100	215.6	6	0.021	282.3	114.4–587.1
	100– < 200	30.8	0	0.003	0	n/a
					− 0.33 ($P=1$)	
ortho-Chloroaniline (OCA) exposure ($n=78$)	All	1367.7	9	0.122	73.7	35.9–135.2
Unlagged	0 < – < 50	1125.9	7	0.101	69.6	30.5–137.7
	50– < 100	191.0	2	0.018	112.1	18.8–370.3
	100– < 200	50.8	0	0.004	0	n/a
					− 0.33 (P = 1)	
para-Toluidine (PT) exposure ($n=18$)	All	146.7	2	0.010	200.0	33.5–660.8
Unlagged	0 < – < 50	107.3	2	0.007	290.9	48.8–961.2
	50– < 100	39.4	0	0.003	0	n/a
					− 1 ($P=1$)	

SIR the standardized incidence ration based on rates for Japan

P analyzed trends using Kendall rank correlation coefficient, *CI* Confidence interval

A limitation of this study was the low follow-up rate; 41.1% of 321 subjects recorded in the company's job history files since 1988.

SIR study

To best of our knowledge, this study is the first analysis of the risk of BCa in Japanese workers exposed to OT. Since no studies to date have monitored OT exposure, we calculated the surrogate exposure levels of each aromatic amine. The SIR for BCa in male workers exposed to OT was 56.8 when compared to the general Japanese male population. Further, it was 363.3 among workers exposed to OT with a surrogate exposure level higher than 300. In addition, a significant positive trend in the SIRs for BCa was noted with the surrogate exposure level of OT in the unlagged, 5-year lagged, and 10-year lagged analyses. In this study, the dose–response relationship was maintained between the estimated exposure of OT and the SIR up to "10-years lagged". Therefore, to investigate the relationship between OT exposure and incidence of BCa, it seemed necessary to observe for at least 10 years, taking into account the latency period.

All patients with BCa had been exposed primarily to OT and co-exposed to MX (IARC group 3), AN (IARC group 3 and group 2A in preparation), OA (IARC group 2B and group 2A in preparation) and OCA (IARC unlisted group) (IARC 2020). Since the surrogate exposure level of OT was higher than that of MX, AN, OA, and OCA in most cases, we concluded that OT was the main agent responsible for BCa (Nakano et al. 2018). A causal relationship between occupational exposure to OT and BCa has been reported in several cohort studies performed in the US, UK,

and Italy. The subjects in these studies had been concurrently exposed to other aromatic amines such as 4-chloro-o-toluidine (IARC group 2A), 4-chloro-acetyl-o-toluidine (IARC unlisted group), indigo (Ott and Langner 1983) (IARC unlisted group), 2-mercaptobenzothiazole (IARC group 2A), phenyl-β-naphthylamine (Sorahan 2008) (IARC group 3), AN (IARC group 3 and group 2A in preparation), nitrobenzene (Carreón et al. 2014) (IARC group 2B) and/or 4,4′-methylene bis (2-methylaniline) (Rubino et al. 1982) (IARC group 2B) (IARC 2020). The SIR in these studies ranged from 2.87 (Carreón et al. 2014) to 5.56 (Sorahan 2008). While the SIR could vary with an exposure dose of OT and the other chemicals, this result was higher than the values of SIR in the previous studies (Carreón et al. 2014; Sorahan 2008).

The SIR for BCa in OT-exposed workers was 56.8 (95% CI 27.7–104.3) in this study. After adjusting for a smoking bias of 1.32–1.69 in the Japanese population, the SIR was still estimated to be high (33.5–42.8). Almost all BCa patients had been engaged in processes of filtering and rinsing the crude product (AAOT) with an organic solvent, drying the rinsed wet product, and packing it (powder). Thus, possible reasons for the high SIR will include subsequent metabolism of the product of OT acetoacetylation. Urinary OT and OT metabolites (Eitaki et al. 2019) in AAOT-administered rats were 10 times higher than those of AAOT and AAOT metabolites, and increased in a dose-dependent manner (Okuno et al. 2019; Yukimatsu et al. 2019). It is possible that exposure reduction of the powdered product was insufficient. In addition, AAOT-promoting effects can induce bladder carcinogenesis more strongly. Jun proto-oncogene (JUN), a transcription factor in the activator protein-1 (AP-1) complex, is involved in numerous cell activities, including proliferation, apoptosis, and differentiation. The expression of JUN and its downstream target genes was increased in the urothelium of male rats (Yukimatsu et al. 2019). Next, OT skin penetration is as fast as β-naphthylamine and reaches maximum penetration rate 4–7 h after the start of exposure (Luersen et al. 2006). Fast transdermal absorbed OT circulates systemically from the capillaries with a dermal metabolism (Korinth et al. 2013), and transfers in part directly from the kidney to the bladder. OT metabolites transported in a stable form to the bladder may have some effects.

The strength of this study was its retrospective nature and the lengthy follow-up of subjects who were hired after 1988 at factory A, where the accumulation of BCa cases occurred. While minimizing the effects of lost cases due to retirement, we evaluated the SIR for BCa based on the exposure to each OT and other aromatic amine at factory A. The study, however, has several limitations. Smoking is one of the most important risk factors for BCa. The bias factor of smoking was estimated to be 1.00 in this study, since the HR for BCa among Japanese males was reported to be 1.32 (95% CI 0.80–2.16) among former smokers, and 1.69 (95% CI 1.09–2.63) among current smokers. Since the additional effects of smoking in the SIR would be 1.32–1.69, the SIR adjusted for smoking was estimated to be 33.5–42.8. Next, we excluded 219 aromatic amine-exposed workers in factories B and C from the cross-sectional analyses. Therefore, the prevalence of bladder lesion-related signs and symptoms of aromatic amine-exposed workers in factory A might be overestimated because the prevalence of excluded workers with lower aromatic amine exposure may be low. Finally, there were limits to the effects of exposure to different combinations of chemicals such as MX, AN and products. However, the results most clearly showed a dose–response relationship between OT and BCa. There was also no evidence of any of the compounds being more carcinogenic those in the IARC (IARC 2020).

Conclusions

We conclude that OT may be responsible for the increased risk of BCa because overall SIRs in OT-exposed workers were significantly high and apparent dose–response relationships between SIRs and surrogate exposure levels were revealed in the 0-, 5- and 10-year lagged analyses. In this study, both BCa patients and non-patient workers exposed to aromatic amines had a high prevalence of a clinical history of cystitis and bladder lesion-related symptoms such as gross hematuria, urination pain, and frequent urination. As these symptoms can be early signs of a bladder tumor, regular monitoring of bladder lesion-related signs and symptoms is essential to identify early-stage BCa in aromatic amine-exposed workers. We will conduct a cohort study to further clarify the relationship between aromatic amines exposure and bladder cancer.

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Author contributions MN and TS contributed equally to this paper. MN and TS takes responsibility for the integrity and accuracy of the

data and drafting of the article. MN, TS, KO, SK, TS, and TT had primary responsibility for the design of study. MN, TS, YE, KO, AT, ST, SK, TS, and TT contributed to analysis and interpretation of data, and KO, TS, and TT assisted in the preparation of the manuscript. MN, YE, KO, ST, SK, and TT contributed to fieldwork management. All the authors assisted in the study design, contributed to data collection and assisted in interpretation of data and to critical revision of the manuscript. All the authors approved the final version of the manuscript. TS's current address is Medical Research and Development Office, Nippon Life Insurance Company, Osaka, Japan.

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Data availability Data are available on request from the authors.

Compliance with ethical standards

Conflict of interest None of the authors has any conflicts of interest to disclose.

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Informed consent to participate Written consent was obtained from all the subjects.

Informed consent for publication Written consent was obtained from all the subjects.

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