The Effect of Smoking on Progression from Ductal Carcinoma In Situ to Invasive Ductal Carcinoma: A Retrospective Study

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Abstract

Background: If ductal carcinoma in situ (DCIS) is diagnosed by histological examination such as core needle biopsy or vacuum-assisted biopsy (VAB), invasion is often found by removing the entire tumor and performing pathological examination. According to a meta-analysis, the rate of invasion found by postoperative pathological examination is about 25%. Smoking is a risk factor for carcinogenesis in various carcinomas, including breast cancer. We examined the correlation between the risk of invasion found by postoperative pathology and smoking history in patients who were diagnosed with DCIS by preoperative biopsy.

Methods: In this study, we examined 128 patients who were diagnosed with DCIS by preoperative biopsy. Before the biopsy, all patients were asked about the duration of smoking, number of cigarettes smoked per day, and whether they were currently smoking. The data were used to calculate each patient's tobacco exposure based on pack-years. The statistical analyses included the Pearson's chi-square test and logistic analysis. Multivariate analysis was performed on the risk factors for invasion diagnosed by postoperative pathological examination in all cases diagnosed with DCIS by preoperative biopsy.

Results: Of all the patients included in this study, 107 (83.5%) never smoked, which represented the majority, while 8 patients (6.3%) smoked at diagnosis with DCIS, and 13 (10.2%) had quit smoking before diagnosis. Tobacco exposure was 10 or less pack-years for 11 patients (8.6%) and more than 10 pack-years for 10 patients (7.8%). Number of pack-years was not an independent factor (p=0.349, odds ratio [OR]=0.329), but current smoker status (p=0.006, OR=not calculable) was an independent factor with VAB (p=0.018, OR=0.327).

Conclusions: This study suggests that tobacco components may have an influence on the progression from DCIS to invasive ductal carcinoma.

Background

If ductal carcinoma in situ (DCIS) is diagnosed by histological examination such as core needle biopsy (CNB) or vacuum-assisted biopsy (VAB), invasion is often found by removing the entire tumor and performing pathological examination. According to a meta-analysis, the ratio of invasive ductal carcinoma (IDC) found by postoperative pathological examination is 25.9% (18.6–37.2%) [1]. Various underestimated risk factors for invasion have been reported, including the grade of DCIS [1]. The natural history of the progression from DCIS to IDC is unknown [9, 10]. However, it is generally believed that the carcinogenesis process for breast cancer progresses from normal breast tissue to atypical ductal hyperplasia, then to DCIS, and lastly to invasive breast cancer [11–13].

Smoking is a risk factor for carcinogenesis in various carcinomas and breast cancer is no exception [2]. Several studies *in vivo* and *in vitro* have shown that tobacco smoke components increase breast cancer cell proliferation and cause malignant transformation [3–5]. In reports investigating whether smoking is a risk factor for DCIS, no correlation was found in a large-scale cohort or case-control studies, while another case-control study showed an inverse correlation between smoking and DCIS [6–8].

There has been no report of smoking as a risk factor to underestimate DCIS by biopsy. However, there are reports about tobacco components reaching the mammary gland tissue through the blood, causing DNA damage [14, 15]. We hypothesized that DCIS may also be affected by smoking components, causing increased malignancy. Based on this hypothesis, we examined the correlation between the risk of invasion found by postoperative pathology and smoking history in patients who were diagnosed with DCIS by preoperative biopsy.

Methods

Patients

In this study, we examined 128 patients who were diagnosed with DCIS by preoperative biopsy from August 2007 to January 2018 at the Osaka City University Hospital. Before the biopsy, all patients were asked about the duration of smoking, the number of cigarettes smoked per day, and whether they were currently smoking. Based on their smoking status, the patients were classified into never-smokers, current-smokers, and former-smokers. The total number of smokers was the sum of current-smokers and former-smokers. The data were used to calculate each patient's tobacco exposure based on pack-years [16] **(Supplementary Table 1)**. Then, all patients were diagnosed with DCIS pathologically by CNB or VAB. The biopsy tissue was used to assess the grade of DCIS based on the World Health Organization classification [17], comedo necrosis, intraluminal calcification, and interstitial inflammation (as previously reported [18, 19]). Immunohistochemical staining was performed in the biopsy tissue to evaluate the expression of the estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki67 in cancer cells. Tumor size was measured by ultrasonography, computed tomography (CT), and magnetic resonance imaging. Cases suspected of invasion or metastasis by these imaging techniques were excluded. About half of all patients did not undergo mammography. All patients underwent mastectomy or breast-conserving surgery after being diagnosed with DCIS.

Statistical analysis

The JMP software package version 15 (SAS, Tokyo, Japan) was used for all statistical analysis. The relationship between each factor was examined by the Pearson's chi-square test. Logistic analysis was used for calculating the odds ratio (OR) and 95% confidence interval. The multivariate logistic regression model was used for multivariate analysis. A p-value lower than 0.05 was defined as significant.

Ethics statement

This study was conducted at the Osaka City University Graduate School of Medicine (Osaka, Japan). The study protocol involved a retrospectively written research, pathological evaluation, and statistical analysis plan. The study complied with the provisions of the Declaration of Helsinki, and all patients provided written informed consent for their treatment and data collection. The study's retrospective protocol was approved by the ethics committee of Osaka City University (approval number #926).

Results

Clinicopathological features

The clinicopathological features of 128 patients diagnosed with DCIS by preoperative biopsy are listed in Table 1. The median age was 51 years (range, 30–78 years), and 17 patients (13.2%) were under 40 years old. Eighty-three patients (64.8%) had some symptoms, and the tumor was palpable at medical consultation in ninety-three patients (72.7%). Forty-five patients (35.2%) were asymptomatic and were found by routine screening for breast cancer or CT examination for other diseases. The median tumor diameter was 17.7 mm (range, 3.0–50.0 mm). For the biopsy method, 73 patients (57.0%) were diagnosed by CNB, which was more than that by VAB. The expression of ER and PgR were positive in 104 (81.3%) and 87 (68.0%) DCIS cases, respectively. Regarding HER2, the score was 2 + in 22 patients (17.2%) and 3 + in 19 patients (14.8%). The expression of Ki67 was higher than 14% in 27 patients (21.1%). Regarding the grade of DCIS, 53 patients (41.4%) had low-grade, 51 (39.8%) had intermediate-grade, and 24 (18.8 %) had high-grade DCIS. In the biopsy specimens, comedo necrosis was found in 68 patients (53.1%), and intraductal calcification was found in 21 patients (16.4%). Lymphoid infiltrate was classified into four stages: Stage1, Stage2, Stage3, and Stage4; it was evaluated as moderate or severe in 39 patients (30.4%). IDC was found by postoperative pathological examination in 50 patients (39.1%).

Table 1
Clinicopathological features of 128 cases diagnosed with DCIS by preoperative biopsy

Parameters	Number of patients (n = 128) (%)
Age at operation (years old)	Median 51 (range; 30–78)
≤40 / 41–60 / 60<	17 (13.2 %) / 72 (56.3 %) / 39 (30.5%)
Symptoms	45 (35.2 %) / 83 (64.8 %)
Asymptomatic / Symptomatic	
Palpability	35 (27.3 %) / 93 (72.7 %)
Impalpabe / Palpable	
Tumor size (mm)	Median 17.7 (range; 3.0–50.0)
≤10 / 10<, ≤ 20 / 20<, ≤ 30 / 30<	28 (21.9 %) / 48 (37.5 %) / 33 (25.8 %) / 19 (14.8 %)
Biopsy device	73 (57.0 %) / 55 (43.0 %)
Core needle biopsy / Vacuum-assisted biopsy	
Estrogen receptor	24 (18.7 %) / 104 (81.3 %)
Negative / Positive	
Progesterone receptor	41 (32.0 %) / 87 (68.0 %)
Negative / Positive	
HER2	87 (68.0 %) / 22 (17.2 %) / 19 (14.8 %)
0,1 / 2 / 3	
Ki67	101 (78.9 %) / 27 (21.1 %)
≤ 14 % / >14 %	
Grade of DCIS	53 (41.4 %) / 51 (39.8 %) / 24 (18.8 %)
Low / Intermediate / High	
Comedonecrosis	60 (46.9 %) / 68 (53.1 %)
Absence / Presence	
Intraductal calcification	107 (83.6 %) / 21 (16.4 %)
Absence / Presence	
Lymphoid infiltrate	33 (25.8 %) / 56 (43.8 %) / 30 (23.4 %) / 9 (7.0 %)
Negative / Mild / Moderate / Severe	
Postoperative pathology	78 (60.9 %) / 50 (39.1 %)
DCIS only / Invasive ductal carcinoma	
Smoking status	107 (83.5 %) / 13 (10.2 %) / 8 (6.3 %)
Never smokers / Former smokers / Current smokers	
Numbers of cigarettes (per day)	107 (83.6 %) / 9 (7.0 %) / 12 (9.4 %)
0 / 0<, ≤ 10 / 10<	
Years of smoking (years)	107 (83.6 %) / 7 (5.5 %) / 9 (7.0 %) / 5 (3.9 %)
0 / 0<, ≤ 10 / 10<, ≤ 20 / 20<	

DCIS: ductal carcinoma in situ, HER2: human epidermal growth factor receptor 2

Parameters	Number of patients (n = 128) (%)
Pack-years of smoking 0 / 0<, ≤ 10 / 10<	107 (83.6 %) / 11 (8.6 %) / 10 (7.8 %)
DCIS: ductal carcinoma in situ, HER2: human epidermal growth factor receptor 2	

Regarding smoking, the majority of patients were never-smokers (107 patients, 83.5%). Eight patients (6.3%) smoked at diagnosis of DCIS (current-smokers), and thirteen patients (10.2%) had quit smoking before diagnosis (former-smokers). Twelve patients (9.4%) smoked more than ten cigarettes per day, which were more than half of the total number of smokers. Regarding the period of smoking, 7 patients (5.5%) smoked for 10 years or less, 9 patients (7.0%) smoked for 11 to 20 years, and 5 patients (3.9 %) smoked for more than 20 years. Tobacco exposure was 10 or less pack-years for 11 patients (8.6%) and more than 10 pack-years for 10 patients (7.8%).

Risk factors for finding invasion in postoperative pathological examination (univariate analysis)

Univariate analysis was performed on clinicopathologic features in which invasion was found in postoperative pathology, and the results are shown in Table 2. IDC tended to be found postoperatively in patients who had symptoms at diagnosis (p = 0.082, OR = 1.980) or whose tumor was palpable (p = 0.058, OR = 2.278). The OR and tumor size were positively correlated, and tumors larger than 30 mm were significantly more invasive than those smaller than 10 mm (p = 0.015, OR = 4.518). DCIS diagnosed by VAB was significantly more invasive than DCIS diagnosed by CNB (p = 0.018, OR = 0.407). Invasion was found more frequently in postoperative pathology in ER negative DCIS than in ER positive DCIS (p = 0.009, OR = 0.304). The relation with PgR was similar but not significant (p = 0.053, OR = 0.476). Regarding HER2, patients with a score of 3 had more invasion than did patients with scores of 0 or 1 (p = 0.027, OR = 3.097). DCIS with high Ki67 had a significantly higher risk of finding IDC than did DCIS with low Ki67 (p = 0.016, OR = 0.304). High-grade DCIS was significantly more likely to have IDC in postoperative pathological diagnosis than did low-grade DCIS (p = 0.007, OR = 3.889). With respect to lymphoid infiltrate, the risk of finding invasion increased as the density of lymphocytes surrounding DCIS increased. As a result, compared with negative-lymphoid infiltrate, invasion was found to be significant in DCIS that had moderate (p = 0.004, OR = 4.606) or severe (p = 0.029, OR = 5.333) infiltrates.

Table 2
Univariate analysis with postoperative pathology

Parameters	Postoperative pathology	Odd ratio	95 % CI	*p* value
	DCIS only / IDC			
Age at operation (years old)	9 (52.9 %) / 8 (47.1 %)	Reference	Reference	0.403
≤40	46 (63.9 %) / 26 (36.1%)	0.636	0.219–1.848	0.675
41–60	23 (59.0 %) / 16 (41.0 %)	0.783	0.249–2.462	
60<				
Symptoms	32 (71.1 %) / 13 (28.9 %)	Reference	Reference	0.082
Asymptomatic	46 (55.4 %) / 37 (44.6 %)	1.980	0.911–4.304	
Symptomatic				
Palpability	26 (74.3 %) / 9 (25.7 %)	Reference	Reference	0.058
Impalpabe	52 (55.9 %) / 41 (44.1 %)	2.278	0.962–5.391	
Palpable				
Tumor size (mm)	21 (75.0 %) / 7 (25.0 %)	Reference	Reference	0.446
≤10	32 (66.7 %) / 16 (33.3 %)	1.500	0.528–4.265	0.059
10<, ≤ 20	17 (51.5 %) / 16 (48.5 %)	2.824	0.945–8.435	0.015
20<, ≤ 30	8 (42.1 %) / 11 (57.9 %)	4.518	1.303–15.660	
30<				
Biopsy device	38 (52.1 %) / 35 (47.9 %)	Reference	Reference	0.018
Core needle biopsy	40 (72.7 %) / 15 (27.3 %)	0.407	0.192–0.862	
Vacuum-assisted biopsy				
Estrogen receptor	9 (37.5 %) / 15 (62.5 %)	Reference	Reference	0.009
Negative	69 (66.3 %) / 35 (33.7 %)	0.304	0.121–0.765	
Positive				
Progesterone receptor	20 (48.8 %) / 21 (51.2 %)	Reference	Reference	0.053
Negative	58 (66.7 %) / 29 (33.3 %)	0.476	0.223–1.016	
Positive				
HER2	56 (64.4 %) / 31 (35.6 %)	Reference	Reference	0.737
0, 1	15 (68.2 %) / 7 (31.8 %)	0.843	0.311–2.289	0.027
2	7 (36.8 %) / 12 (63.2 %)	3.097	1.105–8.676	
3				
Ki67	67 (66.3 %) / 34 (33.7 %)	Reference	Reference	0.016
≤14%	11 (40.7 %) / 16 (59.3 %)	2.866	1.199–6.852	
>14%				

DCIS: ductal carcinoma in situ. IDC: invasive ductal carcinoma. CI: confidence intervals. HER2: human epidermal growth factor receptor 2

Parameters	Postoperative pathology	Odd ratio	95 % CI	*p* value
	DCIS only / IDC			
Grade of DCIS	35 (66.0 %) / 18 (34.0 %)	Reference	Reference	0.778
Low	35 (68.6 %) / 16 (31.4 %)	0.889	0.391–2.019	0.007
Intermediate	8 (33.3 %) / 16 (66.7 %)	3.889	1.400-10.801	
High				
Comedonecrosis	41 (68.3 %) / 19 (31.7 %)	Reference	Reference	0.107
Absence	37 (54.4 %) / 31 (45.6 %)	1.808	0.877–3.728	
Presence				
Intraductal calcification	68 (63.6 %) / 39 (36.4 %)	Reference	Reference	0.171
Absence	10 (47.6 %) / 11 (52.4 %)	1.918	0.747–4.922	
Presence				
Lymphoid infiltrate	24 (72.7 %) / 9 (27.3 %)	Reference	Reference	0.895
Negative	40 (71.4 %) / 16 (28.6 %)	1.067	0.408–2.788	0.004
Mild	11 (36.7 %) / 19 (63.3 %)	4.606	1.585–13.387	0.029
Moderate	3 (33.3 %) / 6 (66.7 %)	5.333	1.095–25.985	
Severe				
Smoking status	63 (58.9 %) / 44 (41.1 %)	Reference	Reference	0.728
Never smokers	7 (53.8 %) / 6 (46.2 %)	1.227	0.386–3.901	0.021
Former smokers	8 (100.0 %) / 0 (0.0 %)	-	-	
Current smokers				
Numbers of cigarettes (per day)	63 (58.9 %) / 44 (41.1 %)	Reference	Reference	0.648
0	6 (66.7 %) / 3 (33.3 %)	0.716	0.170–3.017	0.279
0<, ≤ 10	9 (75.0 %) / 3 (25.0 %)	0.477	0.122–1.863	
10<				
Years of smoking	63 (58.9 %) / 44 (41.1 %)	Reference	Reference	0.928
0	4 (57.1 %) / 3 (42.9 %)	1.074	0.229–5.038	0.266
0<, ≤ 10	7 (77.8 %) / 2 (22.2 %)	0.409	0.081–2.063	0.346
10<, ≤ 20	4 (80.0 %) / 1 (20.0 %)	0.358	0.039–3.312	
20<				
Pack-years of smoking	63 (58.9 %) / 44 (41.1 %)	Reference	Reference	0.781
0	6 (54.5 %) / 5 (45.5 %)	1.193	0.342–4.155	0.053
0<, ≤ 10	9 (90.0 %) / 1 (10.0 %)	0.159	0.019–1.301	
10<				

DCIS: ductal carcinoma in situ. IDC: invasive ductal carcinoma. CI: confidence intervals. HER2: human epidermal growth factor receptor 2

Regarding smoking, no significant difference was found between never-smokers and smokers. However, no current-smokers were found to have IDC by postoperative pathological examination, which was significantly different from never-smokers (p = 0.021, OR = cannot be calculated). As the number of cigarettes smoked per day and the smoking period increased, the OR decreased, but it was not significant. When examined by pack-years, IDC tended to be harder to find in patients with more than 10 pack-years of tobacco exposure than in never-smokers (p = 0.053, OR = 0.159).

Correlations between clinicopathological features and smoking status

The correlations between smoking status and clinicopathological features showed that the number of smokers was significantly higher in younger patients (under 40 years old) than in middle age or older patients (over 40 years old) ($p < 0.001$) (Table 3). Former-smokers and current-smokers were significantly younger than never-smokers ($p = 0.008$, $p < 0.001$ respectively). Current-smokers presented with significantly more symptoms than did never-smokers ($p = 0.029$). However, no other clinicopathological features differed based on smoking status. There was no correlation with any clinicopathological features when divided into two groups with a cutoff value of 10 pack-years.

Table 3
Correlation between smoking status and clinicopathological features

Parameters	Smoker		*p* value	Smoking status		*p* value	Smoking status		*p* value	Pack-years		*p* value
	Never smoker (n = 107)	Smoker (n = 21)		Never smoker (n = 107)	Former smokers (n = 13)		Never smoker (n = 107)	Current smokers (n = 8)		≤ 10 (n = 118)	10 < (n = 10)	
Age at operation (years old)	8 (7.5 %)	9 (42.9 %)	< 0.001	8 (7.5 %)	4 (30.8 %)	0.008	8 (7.5 %)	5 (62.5 %)	< 0.001	14 (11.9 %)	3 (30.0 %)	0.105
≤ 40	99 (92.5 %)	12 (57.1 %)		99 (92.5 %)	9 (69.2 %)		99 (92.5 %)	3 (37.5 %)		104 (88.1 %)	7 (70.0 %)	
> 40												
Symptoms	41 (38.3 %)	4 (19.0 %)	0.091	41 (38.3 %)	4 (30.8 %)	0.596	41 (38.3 %)	0 (0.0 %)	0.029	43 (36.4 %)	2 (20.0 %)	0.296
Asymptomatic	66 (61.7 %)	17 (81.0 %)		66 (61.7 %)	9 (69.2 %)		66 (61.7 %)	8 (100.0 %)		75 (63.6 %)	8 (80.0 %)	
Symptomatic												
Palpability	32 (29.9 %)	3 (14.3 %)	0.142	32 (29.9 %)	3 (23.1 %)	0.609	32 (29.9 %)	0 (0.0 %)	0.069	32 (27.1 %)	3 (30.0 %)	0.844
Impalpabe	75 (70.1 %)	18 (85.7 %)		75 (70.1 %)	10 (76.9 %)		75 (70.1 %)	8 (100.0 %)		86 (72.9 %)	7 (70.0 %)	
Palpable												
Tumor size (mm)	65 (60.7 %)	11 (52.4 %)	0.475	65 (60.7 %)	8 (61.5 %)	0.956	65 (60.7 %)	3 (37.5 %)	0.197	69 (58.5 %)	7 (70.0 %)	0.476
≤ 20.0	42 (39.3 %)	10 (47.6 %)		42 (39.3 %)	5 (38.5 %)		42 (39.3 %)	5 (62.5 %)		49 (41.5 %)	3 (30.0 %)	
> 20.0												
Biopsy device	63 (58.9 %)	10 (47.6 %)	0.341	63 (58.9 %)	8 (61.5 %)	0.854	63 (58.9 %)	2 (25.0 %)	0.062	69 (58.5 %)	4 (40.0 %)	0.257
Core needle biopsy	44 (41.1 %)	11 (52.4 %)		44 (41.1 %)	5 (38.5 %)		44 (41.1 %)	6 (75.0 %)		49 (41.5 %)	6 (60.0 %)	
Vacuum-assisted biopsy												
Estrogen receptor	19 (17.8 %)	5 (23.8 %)	0.516	19 (17.8 %)	4 (30.8 %)	0.260	19 (17.8 %)	1 (12.5 %)	0.705	23 (19.5 %)	1 (10.0 %)	0.460
Negative	88 (82.2 %)	16 (76.2 %)		88 (82.2 %)	9 (69.2 %)		88 (82.2 %)	7 (87.5 %)		95 (80.5 %)	9 (90.0 %)	
Positive												
Progesterone receptor	35 (32.7 %)	6 (28.6 %)	0.710	35 (32.7 %)	4 (30.8 %)	0.888	35 (32.7 %)	2 (25.0 %)	0.652	39 (33.1 %)	2 (20.0 %)	0.396
Negative	72 (67.3 %)	15 (71.4 %)		72 (67.3 %)	9 (69.2 %)		72 (67.3 %)	6 (75.0 %)		79 (66.9 %)	8 (80.0 %)	
Positive												

DCIS: ductal carcinoma in situ, HER2: human epidermal growth factor receptor 2

Parameters	Smoker		*p* value	Smoking status		*p* value	Smoking status		*p* value	Pack-years		*p* value
	Never smoker (n = 107)	Smoker (n = 21)		Never smoker (n = 107)	Former smokers (n = 13)		Never smoker (n = 107)	Current smokers (n = 8)		≤ 10 (n = 118)	10 < (n = 10)	
HER2												
≤2	91 (85.0 %)	18 (85.7 %)	0.937	91 (85.0 %)	10 (76.9 %)	0.449	91 (85.0 %)	8 (100.0 %)	0.238	101 (85.6 %)	8 (80.0 %)	0.633
3	16 (15.0 %)	3 (14.3 %)		16 (15.0 %)	3 (23.1 %)		16 (15.0 %)	0 (0.0 %)		17 (14.4 %)	2 (20.0 %)	
Ki67												
≤14 %	86 (80.4 %)	15 (71.4 %)	0.358	86 (80.4 %)	10 (76.9 %)	0.769	86 (80.4 %)	5 (62.5 %)	0.230	93 (78.8 %)	8 (80.0 %)	0.930
>14 %	21 (19.6 %)	6 (28.6 %)		21 (19.6 %)	3 (23.1 %)		21 (19.6 %)	3 (37.5 %)		25 (21.2 %)	2 (20.0 %)	
Grade of DCIS												
Low, intermediate	85 (79.4 %)	18 (85.7 %)	0.507	85 (79.4 %)	11 (84.6 %)	0.660	85 (79.4 %)	7 (87.5 %)	0.582	93 (78.8 %)	10 (100.0 %)	0.105
High	22 (20.6 %)	3 (14.3 %)		22 (20.6 %)	2 (15.4 %)		22 (20.6 %)	1 (12.5 %)		25 (21.2 %)	0 (0.0 %)	
Comedonecrosis												
Absence	49 (45.8 %)	11 (52.4 %)	0.580	49 (45.8 %)	7 (53.8 %)	0.583	49 (45.8 %)	4 (50.0 %)	0.818	55 (46.6 %)	5 (50.0 %)	0.837
Presence	58 (54.2 %)	10 (47.6 %)		58 (54.2 %)	6 (46.2 %)		58 (54.2 %)	4 (50.0 %)		63 (53.4 %)	5 (50.0 %)	
Intraductal calcification												
Absence	89 (83.2 %)	18 (85.7 %)	0.774	89 (83.2 %)	11 (84.6 %)	0.895	89 (83.2 %)	7 (87.5 %)	0.751	99 (83.9 %)	8 (80.0 %)	0.749
Presence	18 (16.8 %)	3 (14.3 %)		18 (16.8 %)	2 (15.4 %)		18 (16.8 %)	1 (12.5 %)		19 (16.1 %)	2 (20.0 %)	
Lymphoid infiltrate												
Negative, mild	73 (68.2 %)	16 (76.2 %)	0.468	73 (68.2 %)	9 (69.2 %)	0.941	73 (68.2 %)	7 (87.5 %)	0.253	81 (68.6 %)	8 (80.0 %)	0.454
Moderate, severe	34 (31.8 %)	5 (23.8 %)		34 (31.8 %)	4 (30.8 %)		34 (31.8 %)	1 (12.5 %)		37 (31.4 %)	2 (20.0 %)	

DCIS: ductal carcinoma in situ, HER2: human epidermal growth factor receptor 2

Parameters	Smoker		*p* value	Smoking status		*p* value	Smoking status		*p* value	Pack-years		*p* value
	Never smoker (n = 107)	Smoker (n = 21)		Never smoker (n = 107)	Former smokers (n = 13)		Never smoker (n = 107)	Current smokers (n = 8)		≤ 10 (n = 118)	10 < (n = 10)	
Postoperative pathology	63 (58.9 %)	15 (71.4 %)	0.281	63 (58.9 %)	7 (53.8 %)	0.728	63 (58.9 %)	8 (100.0 %)	0.021	69 (58.5 %)	9 (90.0 %)	0.050
DCIS only	44 (41.1 %)	6 (28.6 %)		44 (41.1 %)	6 (46.2 %)		44 (41.1 %)	0 (0.0 %)		49 (41.5 %)	1 (10.0 %)	
Invasive ductal carcinoma												

DCIS: ductal carcinoma in situ, HER2: human epidermal growth factor receptor 2

Comparing former-smokers and current-smokers, former-smokers smoked significantly fewer cigarettes per day (p = 0.027) and had significantly more invasion in postoperative pathology (p = 0.023) **(Supplementary Table 2)**.

Risk factors for finding invasion in postoperative pathological examination (multivariate analysis)

Multivariate analysis was performed on the risk factors for invasion diagnosed by postoperative pathological examination in all cases diagnosed with DCIS by preoperative biopsy (Table 4). Number of pack-years was not an independent factor (p = 0.349, OR = 0.329), but current-smoker status (p = 0.006, OR = not calculable) was an independent factor with VAB (p = 0.018, OR = 0.327).

Table 4
Univariate and multivariate analysis with upstaging preoperatively DCIS to invasive cancer.

	Univarite analysis			Multivarite analysis		
Parameters	Odd ratio	95 % CI	*p* value	Odd ratio	95 % CI	*p* value
Age at operation (years old) ≤ 40 vs > 40	0.685	0.245–1.911	0.468			
Symptoms Asymptomatic vs Symptomatic	1.980	0.911–4.304	0.082	2.809	0.794–10.717	0.110
Palpability Impalpabe vs Palpable	2.278	0.962–5.391	0.058	0.764	0.183–3.124	0.707
Tumor size (mm) ≤ 20.0 vs > 20.0	2.489	1.197–5.173	0.014	2.618	0.979–7.343	0.055
Biopsy device CNB vs VAB	0.407	0.192–0.862	0.018	0.327	0.119–0.828	0.018
Estrogen receptor Negative vs Positive	0.304	0.121–0.765	0.009	0.978	0.195–4.904	0.978
Progesterone receptor Negative vs Positive	0.476	0.223–1.016	0.053	0.882	0.258–3.153	0.843
HER2 ≤2 vs 3	3.203	1.164–8.811	0.020	1.047	0.230–4.652	0.952
Ki67 ≤14 % vs > 14 %	2.866	1.199–6.852	0.016	1.270	0.372–4.241	0.698
Grade of DCIS Low, intermediate vs High	4.508	1.767–11.501	0.001	2.976	0.820-11.504	0.097
Comedonecrosis Absence vs Presence	1.808	0.877–3.728	0.107			
Intraductal calcification Absence vs Presence	1.918	0.747–4.922	0.171			
Lymphoid infiltrate Negative, mild vs moderate, severe	4.571	2.052–10.185	< 0.001	2.419	0.839–7.138	0.102
Smoking status Never smokers vs Smokers	0.573	0.206–1.591	0.281			
Smoking status Never or former smokers vs Current smokers	-	-	0.019	-	-	0.006
Pack-years of smoking ≤10 vs 10<	0.156	0.019–1.275	0.050	0.329	0.014–3.122	0.349

DCIS: ductal carcinoma in situ, CNB: core needle biopsy. VAB: vacuum-assisted biopsy. HER2: human epidermal growth factor receptor 2, CI: confidence intervals

Discussion

There are various reports about risk factors for finding IDC by postoperative pathological examination in cases diagnosed as DCIS by preoperative biopsy. A meta-analysis lists the following items as risk factors: biopsy devices, high-grade DCIS, tumor larger than 20 mm, palpability, and others [1]. There are also reports that list negative hormonal receptor [20, 21], HER2 overexpression [22, 23], and lymphoid infiltrate as risk factors [18, 19]. However, other reports do not identify these clinicopathologic features as risk factors [24]. No study reporting high Ki67 as a risk factor was found, but it was thought that DCIS with HER2 overexpression or high Ki67 had a potential risk of invasion because these features represented a high risk for postoperative DCIS recurrence [25–27].

In this study, the same clinicopathological factors as those previously reported were identified as risk factors for finding infiltration after surgery. However, the patients included in this study differed from the patients of previous reports in terms of clinicopathologic features. An important difference is the rate of IDC found by postoperative pathological examination. In the meta-analysis previously reported, the ratio was 25.9% (18.6–37.2%) [1], while we found a higher rate of 39.1%. We presume that this difference is due to the biopsy device. We have been using 16-gauge CNB because of the physical burden on patients. As a result, IDC was found by postoperative pathological examination at a high-rate of 47.9% in cases diagnosed by CNB, which may have affected the overall rate of IDC found by postoperative pathological examination.

Another difference is that fewer patients had the clinicopathological features listed as risk factors. For example, the palpable tumor rate was 8.8-fold higher in this study compared with a previous report [1]. Similarly, the overexpression rate of HER2 was 14.8% in this study and 28–65% in a previous report [28]. In addition, high-grade DCIS corresponded to 49.4% of cases in a previous report [1] but only 18.8% in this study. These differences may exist due to the low rate of breast cancer screening in Japan, which is lower than that in other countries at 40% [29]. The fact that various risk factors are lower than those reported previously may indicate that, in many cases, invasion already occurred at the time the patient presented to the hospital.

In this study, current-smokers were significantly less likely to find postoperative IDC compared to never-smokers and former-smokers. In addition, the risk of finding IDC decreased with increasing daily smoking and smoking duration, although these were not significant. This is supported by the results of a case-control study. In that study, the current status as a smoker was more important for the onset of DCIS than the exposure to tobacco, and the result was an inverse correlation [8]. However, experiments *in vivo* and *in vitro* have shown that tobacco components increase the malignancy of breast cancer cells [3–5]. In addition, we have shown in clinical samples that smoking can enhance HER2 expression in breast cancer and increase tumor-infiltrating lymphocyte density in the microenvironment surrounding the cancer [16, 30]. From these results, we speculate that smoking may affect DCIS during the beginning of the disease, causing it to acquire invasive ability at an early stage and resulting in IDC identified by biopsy at initial diagnosis. Our speculation is supported by a case-control analysis that listed smoking as a risk of developing luminal A IDC, while it was associated with reduced risk of developing luminal A DCIS; however, the associations were not significant [31].

Some of the limitations of this study are the higher rate of postoperative IDC detection and the fewer number of patients with risk factors than those previously reported. In addition, the data regarding smoking habits may not be entirely accurate because it was based on self-reporting. Because some studies listed younger age as a risk factor for finding invasion by postoperative pathological examination [32–34], the fact that the age distribution was disproportional between smoking and non-smoking patients may represent a small limitation.

Conclusions

This study suggests that tobacco usage may have an influence on the progression from DCIS to IDC, contributing to the elucidation of the underlying mechanism involved in this change.

Abbreviations

CNB: core needle biopsy, CT: computed tomography, DCIS: ductal carcinoma in situ, ER: estrogen receptor, HER2: human epidermal growth factor receptor 2, IDC: invasive ductal carcinoma, OR: odds ratio, PgR: progesterone receptor, VAB: vacuum-assisted biopsy.

Declarations

Ethics approval and consent to participate

A written informed consent to participate in the study was obtained from each subject in accordance with the declaration of Helsinki principles. Each patient or the patient's family was fully informed of the investigational nature of this study and provided their written, informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (approve number #926).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KT participated in the design of the study and drafted the manuscript. SK participated in the design of the study and manuscript editing. YA, WG, RK, AY, SI, and TM helped with study data collection and manuscript preparation. MS, HT, KH and MO conceived the study, and participated in its design and coordination and helped to draft the manuscript. All authors have read and approved the final manuscript.

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