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Research Paper

Use of Underarm Cosmetic Products in Relation to Risk of Breast Cancer: A Case-Control Study

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ABSTRACT

Background: Previous studies on breast cancer (BC), underarm cosmetic products (UCP) and aluminum salts have shown conflicting results. We conducted a 1:1 age-matched case-control study to investigate the risk for BC in relation to self-reported UCP application.
Methods: Self-reported history of UCP use was compared between 209 female BC patients (cases) and 209 healthy controls. Aluminum concentration in breast tissue was measured in 100 cases and 52 controls. Multivariable conditional logistic regression analysis was performed to estimate odds ratios (ORs) with 95% confidence intervals (CIs), adjusting for established BC risk factors.
Findings: Use of UCP was significantly associated with risk of BC ($p = 0.036$). The risk for BC increased by an OR of 3.88 (95% CI 1.03–14.66) in women who reported using UCP's several times daily starting at an age earlier than 30 years. Aluminum in breast tissue was found in both cases and controls and was significantly associated to self-reported UCP use ($p = 0.009$). Median (interquartile) aluminum concentrations were significantly higher ($p = 0.001$) in cases than in controls (5.8, 2.3–12.9 versus 3.8, 2.5–5.8 nmol/g).
Interpretation: Frequent use of UCPs may lead to an accumulation of aluminum in breast tissue. More than daily use of UCPs at younger ages may increase the risk of BC.

1. Background

Breast cancer is the most common cancer in women with a high prevalence in economically developed countries (Kristensen et al., 2014; Parkin et al., 2005). The etiology of breast cancer is multifactorial. Age, genetic mutations and life-time estrogen exposure are well known risk factors (Gail and Pfeiffer, 2015; Petracci et al., 2011; Pfeiffer et al., 2013). These factors explain only a small part of the etiology (Turnbull and Rahman, 2008) suggesting that environmental factors may also be relevant in the development of breast cancer (Bonefeld-Jorgensen et al., 2011; Coyle, 2004). A change in the topological distribution of mammary carcinoma since 1975 (Bright et al., 2016; Darbre, 2016, 2009, 2005, 2003) towards an higher incidence in the upper outer quadrant seems to point to underarm cosmetic products (UCPs) as a potential contributor (Darbre, 2009, 2005, 2003; Darbre et al., 2013b). Previous studies investigating the effect of UCPs on breast cancer have shown conflicting results (McGrath, 2003; Mirick et al., 2002; Pasha et al., 2008; Rodrigues-Peres et al., 2013). Therefore, latest systematic reviews were not able to provide conclusive evidence (Namer et al., 2008; Willhite et al., 2014). Active ingredients in most UCPs are aluminum-based compounds as aluminum chloride and aluminum chlorohydrate. Aluminum salts have been associated with oxidative stress, DNA double strand breaks, proliferation, interference in estrogen action before (Darbre, 2009; Darbre et al., 2013a; Dyrssen et al., 1987; Farasani and Darbre, 2015; Lankoff et al., 2006; Sappino et al., 2012) and with metastasis recently (Mandriota et al., 2016). Mandriota et al. (2016a) demonstrated in an established cancer mouse model that concentrations of aluminum in the range of those measured in human breast are able to transform cultured mammary epithelial cells, enabling them to form tumors and to metastasize. It was further suggested that frequent use of UCPs containing aluminum salts is a main source of measured aluminum in breast structures (Darbre et al., 2013b, 2011; Exley et al., 2007; Mannello et al., 2009). Due to the genotoxic and possibly

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carcinogenic effect of aluminum salts, the use of UCPs may be related to breast cancer (Darbre, 2001; Jennrich and Schulte-Uebbing, 2016; Pineau et al., 2014; Rodrigues-Peres et al., 2013; Sappino et al., 2012).

The relationship of UCPs containing aluminum salts with breast cancer was investigated in few epidemiological studies showing conflicting results (Fakri, 2006; McGrath, 2003; Mirick et al., 2002). Mirick et al. (2002) and Fakri (2006) found no significant associations between antiperspirants and increased risk of breast cancer. In contrast, McGrath (2003) found that patients using UCPs frequently received their breast cancer diagnosis at an earlier age than patients avoiding UCPs. However, none of these studies included breast tissue measurements of aluminum with regard to UCP use. There exists, so far, no controlled study investigating the relationship of aluminum with breast cancer combining an epidemiologic approach with breast tissue measurements.

We conducted a 1:1 age-matched hospital-based case-control study aiming to investigate the risk for breast cancer in relation to self-reported UCP use. We included measurements of aluminum concentrations in breast tissue from a large series of breast cancer patients and healthy individuals in a controlled epidemiologic study. We hypothesized that (1) breast cancer patients had used UCPs more frequently during their lives than healthy controls, that (2) aluminum concentrations in breast tissue is increased in cases, and that (3) there is a relationship between UCP use and measured aluminum concentrations in breast tissue.

2. Methods

2.1. Study Design and Participants

Participants of this age-matched case-control study were recruited between January 2013 and October 2016 at the Medical University of Innsbruck, Austria. Eligible cases were all breast cancer patients aged 20–85 years treated by the Department of Obstetrics and Gynecology who had a confirmed diagnosis of breast cancer within the last 5 years. Eligible controls were women in the same age range (±2.5 years) without a history of malignant breast disease. Controls were recruited either at the Department of Plastic, Reconstructive and Aesthetic Surgery or at other departments. Selection of controls did not follow a formal probability sampling scheme. Because of organizational limitations sampling was done on random time points when trained interviewers were available to find voluntary women fulfilling the inclusion criteria. Cases undergoing mastectomy and healthy controls undergoing reduction mammoplasty were eligible for tissue sampling.

The study was approved by the ethics committee of the Medical University of Innsbruck, (UN4759, 315/4.6). All participants provided their written informed consent before taking part in the study.

2.2. Data Source and Tissue Samples

2.2.1. Structured Personal Interview

A structured personal interview was performed with all study participants by interviewers who were trained to avoid suggestive questions and to use the key words antiperspirants, deodorants and aluminum very carefully. The interviewers were medical school students in their last year and a graduated psychologist. The questionnaire used in these interviews was a modified version of the validated questionnaire used in the MARIE study (Slanger et al., 2007). Study participants were blinded as to the purpose of the study. They were asked to attend a study on life style factors and BC, including questions about nutrition, physical activity and personal hygiene. There was no special focus on UCP use. We also collected information on other BC related characteristics such as estrogen and hormone exposure as well as genetic factors. Questions asked refer to past exposure in four lifetime categories: 'under the age of 30 years', 'between 30 and 50 years', 'over the age of 50 years' and 'last five years before breast cancer diagnoses'. We extended this questionnaire by specific questions regarding personal hygiene, UCP use and aluminum exposure. The majority of UCPs on the market during the past years were antiperspirants containing aluminum salts as active ingredients. There are a few UCPs without aluminum salts commonly called "deodorants" containing ingredients such as perfumes and etheric oils. When asked it turned out that most women were not able to discriminate between these two kinds of UCPs. We therefore concluded that it would be misleading to analyze antiperspirants and deodorants separately and consequently summarized them into the term UCP as the main exposure variable. UCP application categorized in "never", "1–4 times per month", "2–6 times per week", "daily" and "several times per day" was defined as the primary endpoint of this study.

2.2.2. Tissue Sampling and Measurement

Tissue sampling was performed in all cases and controls undergoing surgery. In cases, we took samples of the breast affected by the tumor, in controls sampling was performed on both breasts. Samples of 500 mg were collected near the axilla in the upper outer quadrant, near the mammilla and near the lateral sternal edge in the lower inner quadrant. Thus, we collected three samples in cases and six samples in controls.

In cases, breast tissue was sampled at the day of surgery at the Morphology Laboratory of the Department of Obstetrics and Gynecology during preparation for macroscopic and histo-pathological analysis. In controls, tissue sampling was performed during the breast reduction surgery in the operation theatre of the Department of Plastic, Reconstructive and Aesthetic Surgery. Samples were carefully collected avoiding any background contamination with aluminum regarding the use of surgical instruments, lab tools and vials. Samples were labelled with a patient code blinding any information regarding case/control assignment and tissue location and were immediately frozen and stored at −80 °C at the Department of Biochemistry until analysis. Tissue preparation and defatting was conducted as described in Exley et al. (2007). In brief, thawed tissue was defatted by incubation at 37 °C for maximal 72 h to assure that dried tissue achieved constant weight. Mean of wet weight of samples was 400 mg (±100 mg), mean of dried tissue was 150 mg (±100 mg). Fat was released as clear oil during drying process in inclined plastic weighing boats. For degreasing and tissue transfer we only used metal free instruments. Dry, weighed and defatted tissue was transferred into 20 mL PFA Teflon© vessels with venting plugs and screw caps (CEM Microwave Technology, Germany). Further tissue preparation, digestion and dilution were done according to House et al., 2013. For digestion we used high quality Nitric acid 69% Trace SELECT® (Sigma-Aldrich, Germany). Digested and diluted tissue samples as well as ninety method blanks were analyzed as clear fluids with graphite furnace atomic absorption spectrometer (GF-AAS) with Zeeman–effect background corrector (Thermo Scientific, Germany).

2.3. Statistical Analysis

The sample size of this case-control study was pre-specified and determined to be adequate to detect an odds ratio (OR) of 2 or greater for UCP application on a significance level of 5%.

Assuming a control proportion of 65% UCP use as in Mirick et al. (2002), to achieve 80% statistical power, we were aiming to recruit 200 participants per group, a total of 400 women. In total we recruited 460 participants, 210 cases and 250 controls. Each case was age-matched in a 1:1 ratio to one control subject, minimizing the age difference within case-control pairs by a validated matching algorithm. The application of this algorithm ensured an objective and random assignment of cases to controls in order to reach the optimum result in terms of age difference. Consequently, the pairs differed regarding interview dates.

Patient characteristics, genetic factors, hormone exposure, life style parameters, UCP use were compared between cases and controls using descriptive statistics. Means and medians as well as standard deviations (SD) and interquartile ranges (IQR) were calculated to summarize continuous variables. Categorical variables were presented as frequencies and percentages. We conducted conditional logistic

regression analyses to determine relative risks, estimated as odds ratios (ORs) with 95% confidence intervals (CI) for UCP application and other exposures related to breast cancer. The final multivariable model included all variables that showed a p-value < 0.25 in univariable analyses as well as all relevant variables known to be associated with breast cancer (Pfeiffer et al., 2013). We assessed effect modification through tumor localization and timing of interviews by including interaction terms into the adjusted conditional logistic regression models.

Aluminum concentrations from the different sampling locations (three per case and six per control) were averaged per women, summarized with medians and interquartile ranges (IQR) for cases and controls and stratified by UCP application. In a first step, the summarized aluminum concentrations were compared between cases and controls with an independent t-test. In a second step, a three-way ANOVA for repeated measurements with the between-subject factor 'case versus control', 'UCP use' as ordinal scaled covariate, and the within-subject factor 'sampling location' was performed on $\log10(x + 1)$ aluminum concentrations. We performed subgroup analysis for aluminum measurements separately for cases with tumors in the upper outer quadrant and tumors in other quadrants. We considered a p-value smaller than 0.05 as statistically significant. For both matching and statistical analysis SPSS Statistics v.22 (IBM Analytics, Armonk, NY, USA) was used.

3. Results

A total of 460 women participated in this study, of these 210 were breast cancer cases and 250 were healthy controls. We excluded one case due to breast cancer diagnosis earlier than 5 years before the interview. One control had to be excluded due to unclear breast tissue pathology. Finally, 209 cases were matched 1:1 to 209 controls minimizing the age differences within pairs to a maximum of 3.5 years. Consequently cases and controls did not differ regarding mean age (51.9 ± 12.0 versus 51.8 ± 12.1). Tissue samples were available in 100 cases and 52 controls undergoing surgery.

Characteristics of breast cancer patients and healthy controls together with crude ORs from univariable analyses are shown in Table 1. As expected positive family history of breast cancer was the most pronounced risk factor. Further characteristics that were significantly different between cases and controls were a family history of other cancers such as prostate, ovarian and endometrium cancer, history of benign breast disease and a lower body mass index.

As shown in Table 2, self-reported use of UCP at early ages (<30 years) was significantly associated with an increased risk of breast cancer ($p = 0.0358$) adjusting for age, family history of breast cancer, family history of other cancer, history of benign breast disease, age at menarche, parity, age at birth of first child, age at menopause, menopausal status, hormone replacement therapy, average body mass index and alcohol consumption. This association was triggered by women who reported that they had used UCPs several times per day under their age of 30 increasing their risk for breast cancer by an OR of 3.88 with a 95% CI of 1.03–14.66 ($p = 0.0456$).

Aluminum in breast tissue (Table 3) was found in both cases and controls ranging from 0 to 367.38 nmol/g dry weight and was significantly associated with self-reported UCP use ($p = 0.0344$ for UCP use under the age of 30, $p = 0.0093$ for UCP use during the last 5 years). In cases, median (interquartile) aluminum concentrations observed were 5.8 (2.3–12.9) nmol/g, significantly higher ($p = 0.0014$) than in controls (3.8, 2.5–5.8 nmol/g).

In addition, we analyzed whether tumor localization modifies the relationship between self-reported UCP use, aluminum concentration and the risk for BC. Regarding UCP use there was no significant effect modification by tumor localization ($p = 0.680$ for the UCP use <30 years, $p = 0.341$ for the UCP use during last 5 years). In contrast, regarding measured aluminum concentrations, the stratified results for tumor localization showed significant differences between cases and controls in the subgroup of cases with a tumor in the upper outer quadrant only (Table 4).

4. Discussion

The findings of this age-matched hospital based case-control study suggest an association between UCP use, aluminum concentration in breast tissue and breast cancer. We found a significant difference between cases and controls in the pre-specified primary endpoint.

Table 1
Self-reported characteristics of breast cancer patients and healthy controls.

	Cases ($n = 209$)	Controls ($n = 209$)	Crude OR (95% CI)[a]	p-Value
Age at interview [years, means (SD)]	51·9 (12·0)	51·8 (12·1)		0·2994
Family history of breast cancer (%)	76 (36·4)	32 (15·3)	2·91 (1·81–4·68)	<0·0001
None	133 (63·6)	177 (84·7)	Reference	
1 person	48 (23·0)	27 (12·9)	2·21 (1·30–3·74)	0·0034
2 or more	28 (13·4)	5 (2·4)	6·31 (2·4–6·53)	0.0002
Family history of other cancer (%)	128 (61·5)	103 (49·3)	1·60 (1·09–2·35)	0·0176
History of benign breast disease (%)	63 (30·1)	43 (20·6)	1·61 (1·04–2·48)	0·0326
Age at menarche [years, means (SD)]	13·5 (1·7)	13·4 (1·5)	1·04 (0·92–1·17)	0·5547
Menstruation (%)				
Regular	164 (78·5)	171 (81·8)	Reference	
Unregularly	42 (20·1)	37 (17·7)	1·19 (0·71–1·98)	0·5155
Unknown	3 (1·4)	1 (0·5)		
Hormonal contraceptives (%)	164 (78·5)	168 (80·4)	0·87 (0·52–1·46)	0·5997
Parity (%)	176 (84·2)	172 (82·3)	1·17 (0·68–2·01)	0·5794
Age at birth of first child [years, means (SD)]	26·1 (5·6)	25·1 (5·3)	1·02 (0·98–1·08)	0·3838
Lactation (%)	137 (65·6)	132 (63·5)	1·09 (0·73–1·61)	0·6861
Lactation [months, means (SD)]	3·8 (4·5)	4·0 (5·3)	0·99 (0·95–1·03)	0·7033
Age at menopause	47·3 (7·2)	48·6 (5·7)	0·98 (0·93–1·03)	0·2990
Hormone replacement therapy (%)	44 (21·1)	34 (16·3)	1·42 (0·84–2·39)	0·1881
Average body mass index [kg/m^2, means (SD)]	22·8 (3·4)	23·4 (4·0)	0·95 (0·89–0·99)	0·038
Smoking (%)				
Never	100 (47·8)	98 (46·9)	Reference	
Sometimes	20 (9·6)	28 (13·4)	0·70 (0·37–1·32)	0·2733
Regular	89 (42·6)	83 (39·7)	1·07 (0·69–1·66)	0·7586
Alcohol consumption (%)				
0 drinks per day	29 (13·9)	29 (14·0)	Reference	
≤1 drink per day	172 (82·3)	175 (84·5)	1·02 (0·58–1·82)	0·9364
1+ drink per day	8 (3·8)	3 (1·4)	2·72 (0·65–11·34)	0·1684

[a] Derived from univariable conditional logistic regression analysis.

Table 2
Use of underarm cosmetic products (UCP) in breast cancer (BC) patients and healthy controls.

	Number of cases (%) ($n = 209$)	Number of controls (%) ($n = 209$)	Crude OR (95% CI)	Crude *p*-value	Adjusted OR[a] (95% CI)	Adjusted *p*-value
UCP use in women when they were under the age of 30				0·0951		0·0358
Never	43 (20·6)	46 (22·0)	Reference		Reference	
1–4 times per month	19 (9·1)	26 (12·4)	0·83 (0·40–1·73)	0·6222	0·50 (0·20–1·26)	0·1435
2–6 times per week	26 (12·7)	36 (17·2)	0·87 (0·43–1·75)	0·6930	0·53 (0·23–1·25)	0·1486
Daily	103 (49·3)	89 (42·6)	1·40 (0·79–2·53)	0·2603	1·03 (0·51–2·07)	0·9390
Several times per day	18 (8·6)	9 (4·3)	2·84 (1·02–7·89)	0·0451	3·88 (1·03–14·66)	0·0456
Unknown	0 (0·0)	3 (1·4)				
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls				0·1104		0·0822
Never	25 (12·0)	34 (16·3)	Reference		Reference	
1–4 times per month	24 (11·5)	21 (10·0)	1·67 (0·73–3·81)	0·2211	1·41 (0·49–4·04)	0·5216
2–6 times per week	31 (14·8)	45 (21·5)	0·99 (0·49–2·02)	0·9824	0·59 (0·25–1·40)	0·2338
Daily	109 (52·2)	96 (45·9)	1·70 (0·90–3·21)	0·1046	1·22 (0·56–2·66)	0·6105
Several times per day	20 (9·6)	13 (6·2)	2·63 (1·00–6·87)	0·0492	3·16 (0·90–11·15)	0·0736
Unknown	0 (0·0)	0 (0·0)				

[a] Adjusted for age at interview, age at menarche, parity, age at first live birth, menopausal status, age at menopause, MHT drug therapy, history of breast cancer, history of benign breast disease, family history of other cancer, BMI, alcohol consumption in multivariable conditional logistic regression analysis.

However, the observed association of UCP use with breast cancer was in fact limited to women who reported using UCP's several times a day when they were under the age of 30.

In contrast to our findings, previous epidemiologic studies (Fakri, 2006; Mirick et al., 2002) did not support the hypothesis that UCP use increases the risk for breast cancer. Fakri (2006) examined a very small sample of 54 unmatched cases and 50 controls underpowered to detect realistic effect sizes. In their study UCP use was dichotomous categorized in just two levels, using of UCPs versus no use, which is too imprecise in regard to our results, where a significant association was observed only when women used UCPs several times per day. Similarly, in the much larger study of Mirick et al. (2002), UCP use was measured also in a dichotomous way only. In the study of Mirick et al. (2002) study participants were not asked about UCP use in different life time categories and therefore possible effects of UCP use at younger ages were not detectable. In fact, Mirick et al. (2002) reported antiperspirant use rather than UCP use, however, in the light of our experiences it is unclear how the authors discriminated between deodorant and antiperspirant use. Another important difference between Mirick et al. (2002) and our study exists regarding the birth cohorts of breast cancer patients recruited into the two studies. Breast cancer patients participating in the study of Mirick et al. (2002) were diagnosed in the early 1990′s, on average 20 years earlier than patients in our study. At the time relevant for exposure, approximately between 1940 and 1960, the use of UCPs was less common than 20 years later. UCP use strongly increased in the last four decades and also cultural habits such as shaving of axilla hair became only popular during the late 1980′s in western countries (Darbre, 2009, 2003; McGrath, 2003).

So far, there exist six studies that measured aluminum concentration in breast cancer patients comparing concentrations between benign and malign breast tissues (Exley et al., 2007; House et al., 2013; Millos et al., 2009; Ng et al., 1997; Pasha et al., 2008; Rodrigues-Peres et al., 2013). These studies differed considerably regarding the amount of aluminum found in breast tissue likely because of discrepancies in measurement techniques. Regarding, the analytical approach the measured aluminum concentrations in our cohort were similar to the studies of House et al. (2013) and Rodrigues-Peres et al. (2013).

None of the previous studies sampled control tissue from healthy individuals. Our study included tissue measurements of breast cancer patients and healthy individuals observing a significant difference

Table 3
Median (IQR) of total aluminum concentrations [nmol/g dry weight] in breast tissue samples of cases and controls stratified by underarm cosmetic product (UCP) use.

	Cases	n	Controls	n	*p*-value sampling location	*p*-value UCP use	*p*-value cases vs controls
Median (IQR) of Al^{3+} concentration[a]	5·77 (2·29–12·90)	100	3·77 (2·47–5·78)	52			0·0014
UCP use in women when they were under the age of 30[b]							
Never	3·58 (1·72–9·25)	28	2·74 (1·90–4·21)	11	0·100	0·0344	0·0269
Several times per week	7·77 (4·74–11·40)	9	3·07 (2·75–4·52)	4			
Daily	6·07 (2·21–14·89)	53	4·34 (2·67–6·42)	34			
Several times per day	11·29 (3·62–13·21)	9	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	3·58 (1·72–7·32)	20	3·32 (1·90–4·21)	10	0·251	0·0093	0·0376
Several times per week	7·74 (3·23–11·40)	10	3·07 (2·55–5·86)	6			
Daily	6·07 (2·34–14·89)	57	3·96 (2·54–5·99)	31			
Several times per day	12·10 (3·50–14·68)	12	4·86 (2·51–10·23)	5			

[a] Independent samples *t*-test with log10(x + 1) transformed data.
[b] Three-way analysis of variance with log10(x + 1) transformed data. Repeated aluminum measurements at three different sampling locations (upper outer, mammilla and lower inner breast quadrant) were considered as within-subject factor in the ANOVA.

Table 4
Median (IQR) of total aluminum concentrations [nmol/g dry weight] in breast tissue samples of cases and controls stratified by underarm cosmetic product (UCP) use. Subgroup analyses for cases with tumors in the upper outer quadrant (a) and for cases with tumors in other quadrants (b).

	Cases	n	Controls	n	*p*-value sampling location	*p*-value UCP use	*p*-value cases vs controls
a) Tumor located in the upper outer quadrant							
Median (IQR) of Al^{3+} concentration[a]	7·00 (3·10–16·15)	55	3·77 (2·47–5·78)	52			0·0003
UCP use in women when they were under the age of 30[b]							
Never	3·43 (1·55–9·69)	14	2·74 (1·90–4·21)	11	0·757	0·0116	0·0028
Several times per week	7·71 (4·74–7·77)	5	3·07 (2·75–4·52)	4			
Daily	8·35 (3·19–24·87)	31	4·34 (2·67–6·42)	34			
Several times per day	12·25 (8·56–14·68)	4	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	3·09 (1·55–5·34)	10	3·32 (1·90–4·21)	10	0·916	0·0079	0·0054
Several times per week	7·71 (3·27–7·77)	5	3·07 (2·55–5·86)	6			
Daily	7·69 (3·59–18·41)	32	3·96 (2·54–5·99)	31			
Several times per day	12·90 (3·83–16·15)	7	4·86 (2·51–10·23)	5			
b) Tumor located in other quadrants							
Median (IQR) of Al^{3+} concentration[a]	3·94 (1·90–10·92)	45	3·77 (2·47–5·78)	52			0·2642
UCP use in women when they were under the age of 30[b]							
Never	4·63 (1·90–8·82)	14	2·74 (1·90–4·21)	11	0·017	0·3457	0·3558
Several times per week	11·16 (7·08–16·18)	4	3·07 (2·75–4·52)	4			
Daily	3·48 (1·24–8·99)	22	4·34 (2·67–6·42)	34			
Several times per day	3·62 (3·39–12·91)	5	2·51 (1·86–4·86)	3			
UCP use during last 5 years before BC diagnosis in cases/during last 5 years before interview in controls[b]							
Never	4·63 (1·90–7·91)	10	3·32 (1·90–4·21)	10	0·015	0·1316	0·3731
Several times per week	10·92 (3·23–11·40)	5	3·07 (2·55–5·86)	6			
Daily	3·94 (1·51–9·76)	25	3·96 (2·54–5·99)	31			
Several times per day	3·62 (3·39–12·91)	5	4·86 (2·51–10·23)	5			

[a] Independent samples *t*-test with log10(x + 1) transformed data.
[b] Three-way analysis of variance with log10(x + 1) transformed data. Repeated aluminum measurements at three different sampling locations (upper outer, mammilla and lower inner breast quadrant) were considered as within-subject factor in the ANOVA. *P*-values < 0.0125 (Bonferroni correction for multiple comparisons) indicate statistical significance.

regarding aluminum concentrations. Beyond this, we were able to show a significant association between measured aluminum concentrations in breast tissue and self-reported UCP use suggesting dermal absorption of aluminum salts.

Differences in aluminum concentration between cases and controls were only evident when restricting the analysis to cases with tumors in the upper outer quadrant, supporting the hypothesis of Darbre (2005, Darbre, 2009) that tumors in the upper outer quadrant are affected by the use of UCPs. Results of the questionnaire part, however, do not support this hypothesis. Self-reported UCP use did not differ significantly between cases and controls when considering tumor localization.

Tissue samples of controls showed less variation in aluminum concentrations than samples of breast cancer patients. In ten breast cancer patients, aluminum concentrations over 60 nmol/g up to 367 nmol/g dry weight (15–115 nmol/g wet weight) were observed. Mandriota et al. (2016) and colleges recently showed that aluminum salt concentrations of 100 nmol/g wet weight lead to transformation of in-vitro cultured mammary epithelial cells enabling them to form tumors and metastasis in mouse models. In contrast, aluminum concentration in controls reached a maximum of 24.5 nmol/g dry weight (8 nmol/g wet weight) only.

Our study has several strengths. We combined comprehensive questionnaire data of breast cancer cases and healthy individuals on underarm hygiene habits with data of aluminum concentration in tissue samples. We applied a well-developed and accurate method for aluminum measurement (Exley et al., 2007; House et al., 2013). A standardized sampling procedure, high purity of reagents and a high measurement accuracy minimized background contamination. It is likely that aluminum in breast tissue has a patchy distribution (Exley et al., 2007; House et al., 2013), therefore, we collected multiple tissue samples alongside the transect from upper outer to upper inner quadrant.

Certain limitations of our study need to be discussed. A case-control study is susceptible to recall bias. Self-reporting information may be incomplete or inaccurate and may differ between cases and controls. Younger women may remember in more detail about their specific hygiene habits than elderly women. The mix of incident and prevalent cases in our study may be an additional source of bias. We assessed whether the time span between BC diagnosis and interview date is an effect modifier for the relation of UCP use with risk for BC. Although there is no significant effect modification of the different timing of interviews ($p = 0.282$, for the 'UCP use under the age of 30' model, $p = 0.877$ for the 'UCP use in the last 5 years' model) we cannot rule out any recall issues between incident and prevalent cases.

We tried to reduce reporting and measurement bias by performing personal interviews with well-trained interviewers. The limited sample size of the study leads to relatively small numbers in the sub-categories of the main exposure variable. Though significant, the result concerning UCP use several times per day is based on a few cases only. Furthermore, we cannot exclude a reverse causation effect, meaning that the breast tumor may accumulate aluminum. There are studies that reported higher levels of transition metals in tissue of breast cancer patients (Cui et al., 2007; Ionescu et al., 2006; Romanowicz-Makowska et al., 2011). Although, we matched cases and controls on age, the subgroup for tissue sampling is not age matched. However, in our study, aluminum concentrations did not correlate with age ($r = -0.028$, $p = 0.7291$).

In conclusion, our study provides novel insights and additional evidence regarding a possible role of UCP use and aluminum salts in the etiology of breast cancer. Our findings suggest that frequent use of

UCPs may lead to an accumulation of aluminum in breast tissue. We could show that women who reported to use UCPs several times a day starting at an age under 30 years may even have an increased risk for breast cancer. Until definitive answers about the involvement of aluminum in carcinogenesis of breast cancer, we recommend that particularly women at their younger ages should be careful with the use of UCPs and avoid its excessive use.

Collaborators

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Conflicts of Interest

All authors declared no conflicts of interest.

Author Contributions

HU, NC and CL designed the study and wrote the protocol. CL and HU wrote the first draft of the manuscript. CL conducted interviews, did tissue collection and preparation. CE trained CL for tissue preparation and tissue digestion. HT supervised the tissue analysis. CL did the data management and data analysis with the supervision of HU. NC organized study conduct at the Department of Obstetrics and Gynecology. AS, DG and SS performed macro analysis of breast mastectomies. HF supplied laboratory infrastructures for tissue storage and gave organizational support. ST, TC, DE and MH recruited breast cancer patients and performed mastectomies. TB conducted breast reduction surgeries. EMM, JK, CB, SR, FW, DP, FM, TI recruited healthy controls and did interviews. HHL supplied all laboratory infrastructures for tissue analysis and gave his experienced support. All authors were involved in revision of the final manuscript.

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References

Bonefeld-Jorgensen, E.C., Long, M., Bossi, R., Ayotte, P., Asmund, G., Krüger, T., Ghisari, M., Mulvad, G., Kern, P., Nzulumiki, P., Dewailly, E., 2011. Perfluorinated compounds are related to breast cancer risk in greenlandic inuit: a case control study. Environ. Health 10, 88.

Bright, C.J., Rea, D.W., Francis, A., Feltbower, R.G., 2016. Comparison of quadrant-specific breast cancer incidence trends in the United States and England between 1975 and 2013. Cancer Epidemiol. 44, 186–194.

Coyle, Y.M., 2004. The effect of environment on breast cancer risk. Breast Cancer Res. Treat. 84, 273–288.

Cui, Y., Vogt, S., Olson, N., Glass, A.G., Rohan, T.E., 2007. Levels of zinc, selenium, calcium, and iron in Benign breast tissue and risk of subsequent breast cancer. Cancer Epidemiol. Biomark. Prev. 16, 1682–1685.

Darbre, P.D., 2001. Underarm cosmetics are a cause of breast cancer. Eur. J. Cancer Prev. 10, 389–393.

Darbre, P.D., 2003. Underarm cosmetics and breast cancer. J. Appl. Toxicol. 95, 89–95.

Darbre, P.D., 2005. Recorded quadrant incidence of female breast cancer in Great Britain suggests a disproportionate increase in the upper outer quadrant of Breast. Anticancer Res. 25, 2543–2550.

Darbre, P.D., 2009. Underarm antiperspirants/deodorants and breast cancer. Breast Cancer Res. 11 (Suppl. 3), S5.

Darbre, P.D., 2016. Aluminium and the human breast. Morphologie 100, 65–74.

Darbre, P.D., Pugazhendhi, D., Mannello, F., 2011. Aluminium and human breast diseases. J. Inorg. Biochem. 105, 1484–1488.

Darbre, P.D., Bakir, A., Iskakova, E., 2013a. Effect of aluminium on migratory and invasive properties of MCF-7 human breast cancer cells in culture. J. Inorg. Biochem. 128, 245–249.

Darbre, P.D., Mannello, F., Exley, C., 2013b. Aluminium and breast cancer: sources of exposure, tissue measurements and mechanisms of toxicological actions on breast biology. J. Inorg. Biochem. 128, 257–261.

Dyrssen, D., Haraldsson, C., Nyberg, E., Wedborg, M., 1987. Complexation of aluminum with DNA. J. Inorg. Biochem. 29, 67–75.

Exley, C., Charles, L.M., Barr, L., Martin, C., Polwart, A., Darbre, P.D., 2007. Aluminium in human breast tissue. J. Inorg. Biochem. 101, 1344–1346.

Fakri, S., 2006. Antiperspirant use as a risk factor for breast cancer in Iraq. East Mediterr. Health J. 12, 478–482.

Farasani, A., Darbre, P.D., 2015. Effects of aluminium chloride and aluminium chlorohydrate on DNA repair in MCF10A immortalised non-transformed human breast epithelial cells. J. Inorg. Biochem. 152, 186–189.

Gail, M.H., Pfeiffer, R.M., 2015. Is the benign breast disease breast cancer model well calibrated? J. Clin. Oncol. 33, 2829–2830.

House, E., Polwart, A., Darbre, P.D., Barr, L., Metaxas, G., Exley, C., 2013. The aluminium content of breast tissue taken from women with breast cancer. J. Trace Elem. Med. Biol. 27, 257–266.

Ionescu, J.G., Novotny, J., Stejskal, V., Lätsch, A., Blaurock-Busch, E., Eisenmann-Klein, M., 2006. Increased levels of transition metals in breast cancer tissue. Neuro. Endocrinol. Lett. 27 (Suppl. 1), 36–39.

Jennrich, P., Schulte-Uebbing, C., 2016. Does aluminium trigger breast cancer? J. Sci. Technol. 4.

Kristensen, V.N., Lingjærde, O.C., Russnes, H.G., Vollan, H.K.M., Frigessi, A., Børresen-Dale, A.-L., 2014. Principles and methods of integrative genomic analyses in cancer. Nat. Rev. Cancer 14, 299–313.

Lankoff, A., Banasik, A., Duma, A., Ochniak, E., Lisowska, H., Kuszewski, T., Góźdź, S., Wojcik, A., 2006. A comet assay study reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes. Toxicol. Lett. 161, 27–36.

Mandriota, S.J., Tenan, M., Ferrari, P., Sappino, A.-P., 2016. Aluminium chloride promotes tumorigenesis and metastasis in normal murine mammary gland epithelial cells. Int. J. Cancer 139, 2781–2790.

Mannello, F., Tonti, G. a, Darbre, P.D., 2009. Concentration of aluminium in breast cyst fluids collected from women affected by gross cystic breast disease. J. Appl. Toxicol. 29, 1–6.

McGrath, K.G., 2003. An earlier age of breast cancer diagnosis related to more frequent use of antiperspirants/deodorants and underarm shaving. Eur. J. Cancer Prev. 12, 479–485.

Millos, J., Costasrodriguez, M., Lavilla, I., Bendicho, C., 2009. Multiple small volume microwave-assisted digestions using conventional equipment for multielemental analysis of human breast biopsies by inductively coupled plasma optical emission spectrometry. Talanta 77, 1490–1496.

Mirick, D.K., Davis, S., Thomas, D.B., 2002. Antiperspirant use and the risk of breast cancer. 94, 1578–1580.

Namer, M., Luporsi, E., Gligorov, J., Lokiec, F., Spielmann, M., 2008. The use of deodorants/antiperspirants does not constitute a risk factor for breast cancer. Bull. Cancer 95, 871–880.

Ng, K.H., Bradley, D.A., Looi, L.M., 1997. Elevated trace element concentrations in malignant breast tissues. Br. J. Radiol. 70, 375–382.

Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2005. Global cancer statistics, 2002. CA Cancer J. Clin. 55, 74–108.

Pasha, Q., Malik, S.A., Iqbal, J., Shaheen, N., Shah, M.H., 2008. Comparative evaluation of trace metal distribution and correlation in human malignant and benign breast tissues. Biol. Trace Elem. Res. 125, 30–40.

Petracci, E., Decarli, A., Schairer, C., Pfeiffer, R.M., Pee, D., Masala, G., Palli, D., Gail, M.H., 2011. Risk factor modification and projections of absolute breast cancer risk. J. Natl. Cancer Inst. 103, 1037–1048.

Pfeiffer, R.M., Park, Y., Kreimer, A.R., Lacey, J.V., Pee, D., Greenlee, R.T., Buys, S.S., Hollenbeck, A., Rosner, B., Gail, M.H., Hartge, P., 2013. Risk prediction for breast, endometrial, and ovarian cancer in white women aged 50 y or older: derivation and validation from population-based cohort studies. PLoS Med. 10, e1001492.

Pineau, A., Fauconneau, B., Sappino, A.-P., Deloncle, R., Guillard, O., 2014. If exposure to aluminium in antiperspirants presents health risks, its content should be reduced. J. Trace Elem. Med. Biol. 28, 147–150.

Rodrigues-Peres, R.M., Cadore, S., Febraio, S., Heinrich, J.K., Serra, K.P., Derchain, S.F.M., Vassallo, J., Sarian, L.O., 2013. Aluminum concentrations in central and peripheral areas of malignant breast lesions do not differ from those in normal breast tissues. BMC Cancer 13, 104.

Romanowicz-Makowska, H., Forma, E., Bryś, M., Krajewska, W.M., Smolarz, B., 2011. Concentration of cadmium, nickel and aluminium in female breast cancer. Pol. J. Pathol. 62, 257–261.

Sappino, A.-P., Buser, R., Lesne, L., Gimelli, S., Béna, F., Belin, D., Mandriota, S.J., 2012. Aluminium chloride promotes anchorage-independent growth in human mammary epithelial cells. J. Appl. Toxicol. 32:233. https://www.lipodo.de/stetson-43.

Slanger, T., Mutschelknauss, E., Kropp, S., Braendle, W., Flesch-Janys, D., Chang-Claude, J., 2007. Test-retest reliability of self-reported reproductive and lifestyle data in the context of a German case-control study on breast cancer and postmenopausal hormone therapy. Ann. Epidemiol. 17, 993–998.

Turnbull, C., Rahman, N., 2008. Genetic predisposition to breast cancer: past, present, and future. Annu. Rev. Genomics Hum. Genet. 9, 321–345.

Willhite, C.C., Karyakina, N.A., Yokel, R.A., Yenugadhati, N., Wisniewski, T.M., Arnold, I.M.F.F., Momoli, F., Krewski, D., 2014. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. Crit. Rev. Toxicol. 44, 1–80.