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Analysis of population characteristics related to the total effective xenoestrogen burden: A biomarker of xenoestrogen exposure in breast cancer ☆

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ABSTRACT

To analyse the link between breast cancer and the combined effect of environmental xenoestrogens, we developed, standardised and applied a biomarker of exposure to assess the total effective xenoestrogen burden (TEXB) in human adipose tissue in a case–control study. Environmental oestrogens (TEXB-alpha) are separated from endogenous oestrogens (TEXB-beta), and the combined oestrogenic effect is determined from its proliferative effect (E-Screen assay). The aim of the study was to identify potential confounders, effect modifiers or other covariates associated with higher TEXB levels. In cases, age, family history of breast cancer, lactation experience and smoking were associated with TEXB-alpha. In controls, only age was associated with TEXB-alpha levels. In cases, age, educational level, age at menarche, menopausal status, marital status, lactation experience and smoking were associated with TEXB-beta. In controls, only menopausal status was significantly associated with TEXB-beta levels. In conclusion, TEXB, as a biomarker of exposure, takes account of environmental, dietary, lifestyle, genetic and reproductive factors, which are not usually systematically measured across studies.

1. Introduction

Attempts have been made to explain geographical differences in breast cancer incidence in terms of genetic, reproductive and environmental factors, but a conclusive explanation has yet to be achieved.[1] Spain has a low breast cancer incidence in comparison with other European countries, although it has shown an increase in the past few decades.[2,3] Higher breast cancer risk has been associated with conditions implying a greater lifetime cumulative exposure to oestrogens, e.g. age, early menarche, late menopause, nulliparity, late first full-term pregnancy and lack of breast feeding.[1] Some soci-

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odemographic and lifestyle factors, including alcohol and tobacco consumption, have also been widely considered.[4] Although breast cancer onset cannot be directly attributed individually to any of the above risk factors, the length, extent and window of exposure to oestrogens may play a key role in the vulnerability of the female breast.[5]

Environmental chemicals with hormone mimicking activity, the so-called environmental xenoestrogens, have been found in mammary adipose tissue and their contribution in the aetiology of breast cancer has been postulated.[6] Over the past 20 years, numerous epidemiological studies have addressed the role of organochlorine compounds in breast cancer,[6–23] but an association between exposure to single chemicals and disease onset remains inconclusive. Inconsistencies in results may have been caused by differences in the populations or ethnic groups studied, variability in the sensitivity of chemical analyses or failure to adequately control for potential confounders, effect modifiers or other associated covariates. Chemicals may also interact with environmental, dietary, lifestyle, genetic susceptibility and reproductive factors that are not systematically measured across studies.[24] More importantly, a hypothetical association between organochlorines and breast cancer risk cannot be tested on the basis of individual compound levels, and account must also be taken of possible interactions among the chemicals.[25] Interactions among multiple chemicals and endogenous hormones and their natural ligands may impair the internal homeostasis of the oestrogenic environment of mammary tissue, leading to malignant transformation.

The biomonitoring or the direct measurement of xenobiotics or their metabolites in bodily fluid or tissues to estimate the actual absorbed dose of these compounds has been recommended as an alternative to avoid the unpredictable bias of recall and misclassification in case control studies.[26] In order to facilitate the rigorous testing of the putative link between exposure to xenoestrogens and breast cancer, we developed and standardised a method to quantify exposure to xenoestrogens and to discriminate between endogenous hormones and xenoestrogens by separating natural oestrogens (beta fraction) from more lipophilic xenoestrogens (alpha fraction).[27,28] This method enables assessment of the total effective xenoestrogen burden (TEXB) in human adipose tissue by measuring the combined proliferative effect of accumulated chemicals on MCF-7 human breast cancer cells. Moreover, gas chromotography with electron-capture detection and mass spectrometry was used to confirm the presence of 18 organohalogenated chemicals in the alpha-fraction, i.e. aldrin, dieldrin, endrin, lindane, methoxychlor, HCB, vinclozolin, mirex, *p*,*p*′-DDT, *o*,*p*′-DDT, *o*,*p*′-DDD, *p*,*p*′-DDE, endosulphans I and II, endosulphan diol, sulphate, lactone and ether.[28] Extensive testing has demonstrated that the beta-fraction contains endogenous sex steroids and more polar xenoestrogens that are distinct from those eluted in the alpha-fraction, e.g. sex-steroids, nonylphenol, octylphenol and bisphenol-A.[28] Therefore, the alpha-fraction (TEXB-alpha), which contains no endogenous sex-hormones, can be considered a marker of environmental organohalogenated oestrogenic burden.

Using the above approach, a breast cancer case–control study provided the first demonstration of a significant relationship between breast cancer risk and oestrogenicity due to xenoestrogens. Among women below median body mass index (BMI) (median BMI of 28.6 kg/m^2), those with highest TEXB-alpha levels (>228.51 pM Eeq/g lipid; fourth quartile) had a 3.42-fold significantly greater risk of breast cancer versus those with the lowest levels (<0.47 pM Eeq/g lipid; first quartile). Moreover, on a separate analysis of the results for BMI and menopausal status, women with highest TEXB-alpha levels showed a significantly greater risk of breast cancer versus other sub-groups (OR: 5.67; 95% CI: 1.59–20.21). On the other hand, no association with breast cancer risk was found for TEXB-beta or for TEXB-alpha and -beta combined in either the whole study population or any subgroup. All women in the study had measurable concentrations of at least one of the 18 identified organochlorines. This ubiquity of exposure in the study population hampers the demonstration of an aetiologic role for a given compound, and no single chemical could be positively and significantly associated with the biological effect measured by TEXB-alpha or -beta fractions.[29]

The aim of the present breast cancer case–control study was to identify potential confounders, effect modifiers or other covariates related to the total effective xenoestrogen burden (TEXB-alpha and -beta) in order to elucidate the biological and clinical meaning of the biomarker of exposure assessment.

2. Patients and methods

2.1. *Study subjects*

A detailed description of the study population and methods is reported elsewhere.[29] In brief, the study included 260 incident breast cancer cases and 352 controls matched with cases for age (±3 years) and hospital. Because adipose tissue is the compartment of choice for assessing steady-state body burdens of lipophilic contaminants, we selected controls from among women undergoing surgery for non-cancer-related diseases (65% gall bladder surgery, 20% inguinal hernia or abdominal surgery, 5% varicose vein surgery and 10% other surgery). Additional exclusion criteria for controls were the presence of gynaecological or endocrinal disease, treatment for benign breast disease and implant or breast reduction surgery. Breast adipose tissue from cases was obtained intraoperatively and always before initiation of chemotherapy or radiotherapy.

Biological analyses (E-Screen) were performed on adipose tissue from all study subjects for whom adequate adipose tissue samples were available (198 cases and 260 controls). All procedures were performed in accordance with a protocol approved by the Institutional Ethics Review Boards of participating hospitals. Trained interviewers administered structured face-to-face interviews to patients before surgery on sociodemographic characteristics, reproductive history and fertility, menopausal status, use of exogenous hormones (oral contraceptive or hormone replacement therapy), diet, tobacco and alcohol consumption and family history of breast cancer. In order to test for any selection bias, characteristics of women with both successfully completed questionnaire and adequate tissue sample were compared with those of women with only questionnaire but no adequate sample, who were not included in the final study population; no differences in

questionnaire items for known and suspected breast cancer risk factors were found (Table 1).

All women participating in the study were of Caucasian origin and came from the same geographical area. The Regional Health Service provides universal medical cover in the study area, where there are no private hospitals with Oncology Departments.

2.2. Tumour characteristics

Patients (cases) were classified according to pathology reports based on histological study and tumour size (T), lymph node involvement (N) and metastasis (M). Around 75% of subjects were diagnosed in stages I or II (Table 2) of the disease. Tumours were also classified as oestrogen receptor (ER) and/or progesterone receptor (PR) phenotype by using an enzyme immunoassay technique (EREIA, PgREIA, Abbott Laboratories) (Table 2).

2.3. Laboratory analysis

Adipose tissue from all women ($n = 458$) was collected, coded and sent in batches to the Laboratory of Medical Investigations for analysis. Laboratory personnel were blinded to the status of the women. Bioaccumulative compounds were extracted with hexane from 200 mg of adipose tissue by a previously described method[28] and separated by HPLC. The HPLC method was developed to allow the separation of natural oestrogens (beta-fraction) from more lipophilic xenoestrogens (alpha-fraction) without their destruction. Duplicated dry-pooled alpha- and beta-fractions (eluted from 0 to 11 min and 13–30 min, respectively) were resuspended in charcoal-dextran serum and tested in the E-Screen bioassay for oestrogenicity according to the originally described technique[30] with slight modifications.[31] Each sample was assayed in triplicate with a negative (vehicle) and positive (oestradiol) control in each plate. The proliferative effect of fractions was referred to the maximal effect obtained with oestradiol and transformed into oestradiol equivalent units (Eeq) by reading from a dose–response curve prepared using oestradiol (concentration range from 0.1 pM to 10 nM). Results were expressed as total effective xenoestrogen burden (TEXB-alpha and TEXB-beta) in Eeq per gram of lipid.[28]

Table 1 – Characteristics of the study population

	Women studied ($n = 458$)	Women not included ($n = 154$)
Age (years)	56.0[a]	57.1[a]
Marital status		
Single	5.2%	8.5%
Married	78.0%	80.9%
Widowed/separated	16.8%	10.6%
Educational level		
Illiterate	21.4%	12.8%
Literate	40.8%	53.2%
Primary/secondary	31.9%	25.5%
University	5.9%	8.5%
Number of children	3.5[a]	2.9[a]
Age at first full-term pregnancy (years)	24.3[a]	24.7[a]
Lactation (lifetime accumulated months)		
None	24.5%	
1–10	24.9%	
11–33	25.5%	
⩾34	25.1%	
Postmenopausal	65.7%	
BMI (kg/m^2)	29.0[a]	27.6[a]
First-degree family history of breast cancer	5.9%	6.4%
Tobacco[b]	12.0%	
Alcohol[b]	15.7%	
TEXB-alpha [(Eeq)/g of lipid][c]	39.9	
TEXB-beta [(Eeq)/g of lipid][c]	73.2	

a Arithmetic mean.
b Current drinkers or smokers.
c Geometric mean.

2.4. Statistical analysis

TEXB-alpha and -beta values were converted to their natural logarithms in order to reduce skewness, and geometric mean and median values were calculated as measures of central tendency. Non-parametric one-way ANOVA was used to compare log-transformed TEXB-alpha and beta levels among different categories or levels of the study variables. In variables with an ordinal scale, the Jonckheere–Terpstra test for linear trend was also performed. Associations among continuous variables were assessed with Spearman correlation coefficients, which are based upon rank orders and therefore provide comparable results with both untransformed and transformed variables. A multiple regression model with sequential exclusion was performed to obtain the model that best predicted the TEXB-alpha and TEXB-beta, using the square of the multiple correlation coefficient (R^2) as criterion to select variables for the final model. All variables with an R^2 value of more than 10% in the bivariate analysis were included in the prediction model. All statistical analyses were performed using SPSS statistical software.[32]

3. Results

Mean age was slightly lower in cases than in controls (54.8 versus 56.8; $p = 0.06$) despite the age matching. BMI was also lower in cases (27.3 versus 29.6 kg/m^2; $p < 0.01$). Relationships between variables most frequently considered in breast cancer studies and the oestrogenicity of alpha and beta fractions (TEXB-alpha and TEXB-beta) are shown in Table 3. In cases, median age, lactation experience, family history of breast cancer and tobacco consumption were associated with TEXB-alpha levels. Thus, TEXB-alpha levels significantly decreased with the age of patients ($p < 0.05$), with 2.5-fold higher median TEXB-alpha levels in youngest versus oldest cases. The trend across tertiles was also statistically significant ($p_{tendency} = 0.02$). In controls, only age was associated with TEXB-alpha levels but the decrease with higher age, although significant ($p_{tendency} = 0.02$), was more moderate (1.6-fold) than in cases.

Table 2 – Tumour characteristic of 198 women diagnosed with breast cancer

	n	%
Histology grade		
Infiltrating ductal carcinoma	164	82.8
Medullary carcinoma	2	1.0
Lobular carcinoma	16	8.1
Papillary carcinoma	1	0.5
Colloid carcinoma	3	1.5
Others	12	6.1
TNM stage		
0	7	3.5
1	33	16.7
2	108	54.5
3	38	19.2
4	4	2.0
Unknown	8	4.1
Oestrogen receptor status		
ER+	64	32.3
ER–	65	32.8
Unknown	69	34.8
Progesterone receptor status		
PR+	97	48.9
PR–	32	16.1
Unknown	69	34.8

TEXB-alpha levels were also significantly related to more accumulated months of lactation in cases, although a non-linear pattern was observed, with low levels for women with no lactation experience (reference category), highest levels for those with up to 23 cumulative months of lactation, and intermediate levels for women with a longer lactation. After more than 23 accumulated months of lactation, TEXB-alpha levels were 1.6-fold lower than in women with less lactation experience.

A first-degree family history of breast cancer was significantly associated with TEXB-alpha in cancer cases ($p < 0.02$). Finally, tobacco consumption, measured qualitatively (current/former/non-smoker), was significantly associated with TEXB-alpha. Women who had never smoked showed lower TEXB-alpha median values compared with current and former tobacco users ($p = 0.03$).

TEXB-beta levels in cases were associated with age, educational level, age at menarche, menopausal status, marital status, accumulated lactation experience and tobacco consumption. Only menopausal status was significantly associated with TEXB-beta levels in controls, although age and history of employment in agriculture also showed a borderline association. Thus, TEXB-beta levels significantly decreased with the age of patients ($r_s = -0.23$, $p < 0.01$) and were more than 5-fold higher in the youngest versus the oldest women; the trend across all age tertiles was also statistically significant ($p_{tendency} < 0.01$). Age at menarche was also significantly associated with TEXB-beta in cases, with markedly lower levels in women in first ($\leqslant$11 years) versus third ($\geqslant$14 years) tertile, and a significant positive trend across all tertiles ($p_{tendency} = 0.01$). In both cases and controls, menopausal status was associated with TEXB-beta, with higher mean levels in premenopausal versus postmenopausal women and with marital status, with lower levels in single than in married or widowed/separated women. A strong association ($p < 0.01$) was found between TEXB-beta and lactation experience, although a non-linear pattern was observed as in the case of TEXB-alpha. After more than 23 accumulated months of lactation, TEXB-alpha levels were 3-fold lower than in women with less lactation experience.

When tobacco use was considered qualitatively, it was significantly associated with TEXB-beta levels ($p = 0.01$), and current and former smokers showed higher TEXB-beta values compared with women who had never smoked. Finally, educational level was associated with TEXB-beta values, with lower levels in women with no education versus those with some schooling, and a significant trend across the different categories ($p_{tendency} < 0.01$).

All the characteristics of the study population (included in Table 3) were considered in a multivariate regression analysis to assess their predictive capacity. Predictors of TEXB-alpha levels in cases were first-degree family history of breast cancer ($\beta = 0.89$; $p = 0.05$) and former alcohol consumption ($\beta = -1.31$; $p = 0.02$) ($R^2 = 0.41$; $p < 0.01$). In the same model, age (52–62 years: $\beta = -0.74$, $p = 0.02$; >62 years: $\beta = -1.51$, $p < 0.01$), age at menarche (12–13 years: $\beta = 0.89$, $p = 0.03$), marital status (married: $\beta = 0.94$, $p = 0.05$; widowed/separated: $\beta = 1.37$, $p = 0.02$) and alcohol use (former: $\beta = 1.3$, $p = 0.01$; current: $\beta = -0.9$; $p < 0.01$) were the main predictors of TEXB-beta levels ($R^2 = 0.47$; $p < 0.01$). In both models, a strong association was found between TEXB-alpha and TEXB-beta levels (Table 4).

4. Discussion

This breast cancer case–control study allowed us to report the first demonstration of a significant relationship between breast cancer risk and the combined effect of organohalogenated xenoestrogens.[29] In the study population, the total effective xenoestrogen burden (TEXB-alpha) among cases was lower in older (>63-year-olds) versus younger (<52-year-olds) women and in women with more than 23 cumulative months of lactation versus those with less. TEXB-alpha was higher in women with first-degree family history of breast cancer and with former/current smoking habits than in those without. These correlations are of major interest since they can help to explain the biological meaning of TEXB, supporting the utility of biomarkers of xenoestrogen exposure. Unfortunately, the absence of studies using a similar approach impedes comparisons and hampers the quest for explanations of the relationships found.

Controls were selected from among women who underwent abdominal surgery for non-cancer-related diseases, such as gall bladder and hernia, excluding those with gynaecological or endocrinal disease and those undergoing surgery for benign breast disease, implant or breast reduction. Adipose tissue, the compartment of choice for assessing steady-state body burdens of lipophilic contaminants, was intraoperatively obtained from all subjects and before initiation of any anti-tumour treatment (chemotherapy or radiotherapy) in cases. Several case–control studies using adipose tissue from sites other than breast for exposure assessment in controls[17,18,33] have suggested that either abdominal or

Table 3 – Relationship between characteristics of cases/controls and total effective xenoestrogen burden (TEXB α and β) in adipose tissue samples

	Cases (n = 198)							Controls (n = 260)						
	n	TEXB[a] α	P_{K-W}[b]	P_{tend}[c]	TEXB[a] β	P_{K-W}[b]	P_{tend}[c]	n	TEXB[a] α	P_{K-W}[b]	P_{tend}[c]	TEXB[a] β	P_{K-W}[b]	P_{tend}[c]
Age (years)														
⩽52.10	79	88.50	0.05	0.02	211.60	<0.01	<0.01	86	46.52	0.04	0.02	151.39	0.07	0.02
52.11–63.31	65	53.50			67.20			86	49.27			102.75		
⩾63.32	54	35.30			38.36			88	28.67			67.45		
BMI (kg/m^2)														
⩽27.40	107	60.50	0.24	0.45	96.66	0.28	0.61	86	32.07	0.09	0.08	79.45	0.17	0.18
27.41–31.57	46	86.00			229.05			86	31.13			76.50		
⩾31.58	45	40.30			57.50			88	70.05			158.95		
Rural habitat (% years)														
0	86	76.90	0.98	0.74	123.25	0.42	0.38	95	53.75	0.16	0.12	122.50	0.14	0.17
>0 ⩽ 93	63	62.50			103.35			81	43.90			124.50		
>93	49	47.00			62.00			82	24.50			61.82		
Marital status														
Single	17	59.65	0.17	0.64	18.16	0.04	0.68	7	3.55	0.51	–	1.80	0.08	–
Married	152	79.50			125.00			205	41.00			96.50		
Widowed/Separated	29	26.15			60.00			48	47.00			120.90		
Educational level														
Illiterate	30	27.65	0.46	0.29	47.47	0.02	0.01	68	30.50	0.61	0.59	84.50	0.81	0.57
Write and read	80	71.25			78.97			107	36.27			109.50		
Secondary and university	88	27.65			71.25			85	31.00			36.30		
Occupation														
Housewife	50	65.50	0.84	0.63	78.75	0.27	0.13	91	37.10	0.59	0.35	95.50	0.65	0.50
CON groups 4–9	120	61.50			97.88			153	39.55			110.00		
CON groups 1–3	28	91.75			210.55			16	83.75			104.07		
Agriculture														
Yes	110	60.50	0.44	–	11.25	0.57	–	134	45.25	0.76	–	78.70	0.06	–
No	88	79.50			94.35			126	36.27			121.00		
Social status														
4–5	30	91.75	0.43	0.50	253.95	0.13	0.22	25	26.00	0.88	0.88	30.31	0.12	0.16
3	31	43.90			86.00			25	49.30			100.00		
2	41	122.50			102.00			50	60.88			176.00		
1	96	54.87			79.00			160	33.07			98.0		
Number of full-term pregnancies														
0–1	35	60.00	0.45	0.64	83.05	0.20	0.61	24	39.85	0.45	0.45	75.07	0.43	0.52
2–3	110	82.80			132.50			130	44.27			114.75		
⩾4	53	40.30			59.00			106	36.27			91.82		
Age first full-term pregnancy														
⩽19	17	95.00	0.26	0.31	161.45	0.42	0.27	32	65.25	0.48	0.23	130.90	0.88	0.90
20–25	80	60.25			99.33			138	42.22			96.25		
⩾26	76	70.25			114.75			73	36.25			88.15		

Lactation (accumulated months)														
0	53	20.50	0.04	0.32	39.95	0.01	0.35	59	27.50	0.30	0.61	61.00	0.33	0.54
1–23	93	88.50			185.00			97	49.00			122.05		
⩾24	52	55.05			65.36			104	41.27			91.82		
Age at menopause														
⩽44	20	71.25	0.47	0.58	60.50	0.17	0.45	32	24.52	0.49	0.27	75.75	0.63	0.36
45–49	37	84.00			125.00			63	49.30			78.50		
⩾50	141	53.50			106.00			165	43.90			120.00		
Age of menarche														
⩽11	32	79.50	0.44	0.47	148.00	0.04	0.01	62	22.62	0.56	0.92	119.75	0.73	0.58
12–13	103	74.80			132.00			106	63.00			96.25		
⩾14	63	53.50			55.00			92	36.70			82.25		
Contraceptives														
Yes	60	72.75	0.65	–	125.97	0.20	–	64	67.00	0.60	–	150.00	0.10	–
No	138	63.07			97.20			196	37.25			86.35		
Menopausal status														
Premenopausal	80	82.50	0.16	–	195.00	0.05	–	74	43.27	0.13	–	176.00	0.01	–
Postmenopausal	118	63.07			64.60			186	39.25			80.00		
Hormonal replacement therapy														
No	189	62.50	0.85	–	103.35	0.36	–	241	41.00	0.92	–	96.50	0.87	–
Yes	9	79.00			146.50			19	77.95			165.00		
Family history of breast cancer														
Yes	21	130.00	0.02	–	193.00	0.25	–	6	135.97	0.63	–	63.60	0.58	–
No	177	60.00			96.66			254	40.27			103.50		
Tobacco														
Never	150	51.55	0.03	0.01	79.22	0.01	<0.01	223	37.10	0.44	0.28	98.50	0.43	
Former	33	135.50			185.00			22	58.00			85.00		
Current	15	130.00			126.95			15	49.00			122.50		
Alcohol														
Never	144	73.65	0.24	0.24	107.00	0.48	0.48	217	38.95	0.96	0.96	100.00	0.97	
Former	13	64.50			193.00			12	93.10			108.40		
Current	41	60.50			50.00			31	53.75			100.00		

CON, classification of occupation.
a pM Eeq/g lipid, median.
b Probability in Kruskal–Wallis one-way ANOVA.
c Probability in linear tendency test (Jonckheere–Terpstra).

Table 4 – Predictors of TEXB α and TEXB β

Variables	β	SE*	P	
	Ln TEXB α Cases			
Constant	1.01	0.30	0.01	$R^2 = 0.41$
Alcohol				
Never	1			
Former	−1.31	0.58	0.02	
Current	0.16	0.36	0.65	
Family history of breast cancer	0.89	0.47	0.05	
Ln TEXB β	0.64	0.06	<0.01	
	Controls			
Constant	1.07	0.25	<0.01	$R^2 = 0.32$
Ln TEXB β	0.56	0.05	<0.01	
	Ln TEXB β Cases			
Constant	1.61	0.60	<0.01	$R^2 = 0.47$
Age				
<52	1			
52–62	−0.74	0.32	0.02	
>62	−1.51	0.37	<0.01	
Alcohol				
Never	1			
Former	1.3	0.55	0.01	
Current	−0.9	0.34	<0.01	
Age at menarche				
<44	1			
44–49	0.89	0.41	0.03	
>50	0.42	0.30	0.17	
Marital status				
Single	1			
Married	0.94	0.49	0.05	
Widowed/separated	1.37	0.57	0.02	
Ln TEXB α	0.58	0.05	<0.01	
	Controls			
Constant	0.42	0.80	0.59	$R^2 = 0.36$
Employment in agriculture	0.62	0.26	0.02	
Marital status				
Single	1			
Married	1.61	0.81	0.05	
Widowed/separated	1.74	0.86	0.04	
Ln TEXB α	0.57	0.05	<0.01	

* Standard error.

breast adipose tissues can be used to measure body burdens of persistent lipophilic contaminants.

The mean BMI of controls was higher than that of cases, which may be a possible weakness of our study, suggesting a selection bias. In fact, however, 92% of women in the same age range and residence area (Southeast Spain) were reported to be overweight (BMI > 25 kg/m^2) by the European Prospective Investigation into Cancer and Nutrition (EPIC).[34]

We had expected to find a positive association between age and TEXB-alpha, assuming that longer exposure would result in higher levels of bioaccumulable xenoestrogens. In this regard, several studies[35–37] have reported a positive relationship between age and tissue organochlorine levels, although this is not a constant finding by all authors.[38,39] In the present study, TEXB-alpha significantly decreased with age in both cases and controls (oldest women had 2.5-fold and 1.6-fold lower levels than youngest in cases and controls, respectively).

A decrease in TEXB-alpha with age may be an effect of pregnancy and lactation, which may act as a cleaning mechanism of bioaccumulated xenobiotics. In fact, breastfeeding was reported to significantly reduce the maternal burden of environmental toxicants at the expense of increasing the exposure of the child,[40] with the most important clearance of fat-soluble xenobiotics occurring during pregnancy and lactation of the first baby.[41,42] In the present series, lactation experience of more than 23 accumulate months was significantly accompanied by a significantly lower TEXB-alpha.

Among the breast cancer patients, TEXB-alpha was higher in those with a first-degree family history of breast cancer,

and this association persisted after adjustment for age. This finding may be explained either by genetic background (e.g. xenobiotic metabolic profile) or by a similar pattern of exposure for the women and their relatives.[43–45] Further prospective studies are required to explain these results.

Finally, TEXB-alpha was associated with smoking. We found higher levels in women who declared this habit, and this significant difference persisted after adjustment for age, confirming smoking as a plausible source of xenoestrogen exposure.[46–48] However, this finding should be interpreted with caution due to the small number of current and former smokers in the series.

TEXB-beta was inversely related to age and menopausal status in both cases and controls. Age is well known to have a dramatic effect on the level of endogenous oestrogens in women.[49] The simplest explanation is that natural endogenous oestrogens are less abundant in adipose tissue after menopause. On the other hand, endogenous oestrogens are produced in the fat of both pre- and postmenopausal women by conversion of precursors (C-19 steroids) via aromatase cytochrome P450.[50] Adipose tissue plays an important role in the storage and regulation of oestrogen in pre-menopausal women, who were reported to have 3-fold higher concentrations of oestradiol esters (eluted in the beta fraction) in adipose tissue versus postmenopausal women,[51] in agreement with our findings.

Age may also explain associations found between TEXB-beta and some variables such as menopausal status or tobacco consumption, since the influence of these characteristics disappeared in the age-adjusted model (data not shown). However, the relationship between TEXB-beta and other characteristics, i.e. educational level, age at menarche, lactation, marital status and contraceptive use, persisted after age adjustment. We have no explanation for some of these effects, but it is likely that the low number and sparse distribution of subjects in some of the categorical variables (e.g. marital status and tobacco) may be responsible. However, there are plausible explanations for other associations. Thus, the presence of synthetic oestrogen in adipose tissue extracts of women using contraceptive hormones explains the elevated TEXB-beta levels found, since residues of synthetic oestrogens elute together with natural oestrogens and more polar xenoestrogens in the beta fraction.[28]

Although TEXB-beta did not emerge as a risk factor for breast cancer in our case–control study,[29] it is the result of interaction among endogenous and more polar xenoestrogens and also contributes to the total environmental oestrogen burden. Our method efficiently extracts organohalogenated xenoestrogens with a high recovery rate and separates them from ovarian oestrogens. However, this protocol, designed to favour the extraction of bioaccumulable lipophilic xenoestrogens, may not be so effective to extract endogenous sex steroids and more polar xenoestrogens, such as nonylphenol, octylphenol and bisphenol-A. A significant part of these can be lost during the extraction process, contributing to an underestimation of the oestrogenicity of the beta fraction. In the present study, a statistically significant association was found between TEXB of the alpha and beta fractions ($r_s = 0.61$: $p < 0.01$), suggesting a common pattern of exposure to persistent and polar xenoestrogens in the same individuals.

In the multivariate analyses, alcohol use and first-degree family history of breast cancer were the sole predictors of TEXB-alpha values in cases, although this model showed a low predictive power ($R^2 \cong 0.41$). Age disappeared as a predictor factor for TEXB-alpha levels. The predictive factors for TEXB-beta values were age, alcohol use, age at menarche and marital status, and the model showed a slightly higher predictive power ($R^2 \cong 0.47$). TEXB-alpha and TEXB-beta were positively correlated in all multivariate analysis models, again indicating a similar pattern of exposure to non-polar (alpha fraction) and polar xenoestrogens (beta fractions) in some individuals.

Most epidemiological studies in breast cancer have focussed on the impact on disease outcomes of single chemicals. We propose an alternative approach, studying the combined effect of breast adipose tissue extracts via estimation of the oestrogenic burden due to xenoestrogens. The clinical and biological associations reported in this paper support the utility of TEXB as a biomarker of exposure and effect. Future research into endocrine disruption will benefit from this type of approach, in which the effect of mixtures of xenoestrogens can be evaluated.

Conflict of interest statement

None declared.

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