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RESEARCH ARTICLE

Association of breast adipose tissue levels of polychlorinated biphenyls and breast cancer development in women from Chaoshan, China

Yuanfang He[1] · Lin Peng[2] · Yiteng Huang[3] · Xiaodong Peng[1] · Shukai Zheng[1] · Caixia Liu[1] · Kusheng Wu[1]

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Abstract Polychlorinated biphenyls (PCBs) are implied to be potential risk factors for breast cancer in wildlife and in in vivo and in vitro studies. Epidemiological studies revealed some individual or groups of PCB congeners associated with breast cancer risk, but consistent conclusions are scarce. This study aimed to explore the association between PCB exposure and breast cancer development. Breast adipose tissues were collected, and seven PCB congeners were analyzed by gas chromatography–mass spectrometry (GC-MS). Demographic characteristics, basic clinical data, and pathological diagnosis information were obtained from medical records. The differences in PCB exposure levels among different groups and indices were compared, and the correlation among PCB congeners was evaluated. The order of congener profile by molar concentration was PCB-153 > PCB-138 > PCB-180 > PCB-118 > PCB-101 > PCB-52 > PCB-28. ∑PCB level differed by occupation and residence and was significantly higher at 55–59-year-old group than at the other age groups. ∑PCB level was higher in postmenopausal than in premenopausal women. Decreasing ∑PCB levels were related with increasing parity among women with progesterone receptor (PR)-positive breast tumors. With increased clinical stage, the ∑PCB level increased significantly. ∑PCB level did not differ by tumor–node–metastasis classification and PR or human epidermal growth factor receptor 2 (HER2) expression but did differ by estrogen receptor (ER) expression ($P = 0.04$) without a regularly increasing trend in breast adipose tissue. These results suggest a potential association between PCB exposure and breast cancer development. Further in vitro and in vivo studies are needed to confirm these findings and explain the underlying mechanisms.

Keywords Polychlorinated biphenyls · Breast adipose · Exposure · Breast cancer

Yuanfang He and Lin Peng contributed equally to this work.

Highlights
- PCB exposure for women in Chaoshan, China, still remains high.
- PCB-153 was the dominant congener, followed by PCB-138, PCB-180, PCB-118, PCB-101, PCB-52, and PCB-28.
- PCB exposure was significantly associated with breast cancer clinical stage.
- PCBs was related to ER expression in a non-monotonic dose–response way.

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✉ Kusheng Wu
kswu@stu.edu.cn

[1] Department of Preventive Medicine, Shantou University Medical College, No. 22, Xinling Rd, Shantou, Guangdong 515041, China

[2] Clinical Laboratory, Cancer Hospital of Shantou University Medical College, Shantou, Guangdong 515041, China

[3] Health Care Center, The First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong 515041, China

Introduction

Polychlorinated biphenyls (PCBs), comprising 209 different congeners, are industrial man-made compounds in the form of mixtures called Aroclors. PCBs were used widely not only for capacitors and transformers in industry but also in daily life for inks, paints, adhesives, fluorescent light fixtures, carbonless copy paper, plasticizers, and so on (Shields 2006). PCB production was banned first in Western Europe and North America in the late 1970s and in other parts of the world in the 1980s and China in 1983 (Negri et al. 2003; Shields 2006).

However, PCBs still exist ubiquitously in the environment, including atmosphere, dust, rivers, food, soil, fish, wildlife, and animals. PCBs have been found in human hair, adipose tissue, blood, and breast milk at quite high levels (Noakes et al. 2006; Rudel et al. 2008; Syed et al. 2013; Wu et al. 2011; Zheng et al. 2016). Because of their liposoluble characteristic and highly stable chemical properties, they are largely accumulated and persist in the food chain and then enter the human body by ingestion. Human exposure to PCBs occurs mainly not only by food consumption but also by respiratory and dermal exposure (Gasull et al. 2010; Rudel et al. 2008). PCBs are resistant to metabolism and are lipophilic. Once they bioaccumulate in the human body, their excretion is difficult because of a long intrinsic human elimination half-life that is approximately 10 to 15 years (Ritter et al. 2011).

Since the early 1990s, PCBs, as a kind of environmental endocrine-disrupting chemical with estrogenic and antiestrogenic effects, have been suspected as a risk factor of breast cancer (Parent et al. 2011; Ptak et al. 2011; Recio-Vega et al. 2011; Zhang et al. 2015). PCBs are persistent exogenous pollutants and similar to endogenous estrogen in structure and function, so they can bind with the estrogen receptor (ER) in cell in the hypothalamus, pituitary, and uterus tissue, thereby interfering with the combination of endogenous estrogen. Meanwhile, they also affect the process of the normal hormone's release, transport, metabolism, and combination, thus disrupting the endocrine system (Huang et al. 2015; Abdelrahim et al. 2006). Moreover, PCBs may have neural toxic responses and they mediate and induce the neuroendocrine system to disorder the normal control of reproductive development (Bonefeld-Jorgensen et al. 2014; Grandjean and Landrigan 2014; Parent et al. 2011). Therefore, PCB exposure is considered a risk factor of many endocrine system diseases, especially breast cancer (Bonefeld-Jorgensen et al. 2014).

The incidence of breast cancer has been growing every year from the last decades. Breast cancer has become the most familiar female cancer and is also the primary cause of cancer death for females (Dubey et al. 2015; Zeng et al. 2014). In 2012, the rate in China was 30.4/100,000, lower than 71.1/100,000 in Europe (Chen et al. 2016; Dubey et al. 2015). Breast carcinogenesis involved a complex process influenced by many factors. High-penetrance or moderate-penetrance variants in *BRCA1*, *BRCA2*, *PALB2*, *ATM*, and *CHEK2* are identified to contribute to the familial susceptibility to breast cancer (Hall et al. 2013; Rudolph et al. 2016; Mavaddat et al. 2010). Diet, smoking, and alcohol are also contributions to breast cancer risk (Salehi et al. 2008; Miller et al. 2007; Barnard et al. 2015). Most risk factors of estrogen-mediated breast cancer are thought to act by modification of estrogen levels. Hormonal and reproductive factors and exogenous estrogens, such as organochlorine contaminants, oral contraceptives, and hormone replacement therapies, are known to affect breast cancer development (Barnard et al. 2015; Salehi et al. 2008; Trichopoulos et al. 2008). Therefore, hereditary, genetic, reproductive, hormonal, and environmental factors as well as lifestyle and preexisting breast conditions can influence breast cancer development. The environmental factors are the main underlying risk for breast cancer origin, by disrupting the function of endogenous hormones or gene–environment interaction. Natural or artificial xenoestrogenic compounds may play a synergistic effect with endogenous hormones, leading to attached and harmful effects on breast gland (Buterin et al. 2006).

PCBs and breast cancer have been studied widely. Researchers in Europe and the USA have performed numerous epidemiological studies in the past decades, including prospective and retrospective studies. Some studies reported that high levels of PCB-118, PCB-138, PCB-153, and PCB-180 were associated with increased breast cancer risk (Aronson et al. 2000; Muscat et al. 2003; Recio-Vega et al. 2011). A recent meta-analysis showed that group II and group III PCBs (by Wolff classification) were associated with elevated breast cancer risk (Zhang et al. 2015). Nevertheless, Itoh et al. (2009) reported an opposite result that PCBs were protective factors of breast cancer, and most of epidemiological studies found no association between PCB and breast cancer (Negri et al. 2003; Gatto et al. 2007; Bonefeld-Jorgensen et al. 2011). Although epidemiological studies revealed some individual or groups of PCB congeners associated with breast cancer risk, consistent conclusions are scarce.

Previous epidemiological studies generally evaluated the relationship between human blood/serum PCB levels and breast cancer risk, but a handful of studies used breast adipose tissue. Blood/serum PCB levels reflect only recent human PCB exposure but not earlier and specific time exposure. As well, blood volume and lipids are both easily affected by diet, so whether PCB levels are adjusted by lipids or not, they are susceptible to the fluctuation of diet. However, breast adipose PCBs were supplemented from other parts of the body by blood circulation, so their contents are relatively stable and are a combination of both recent and past exposures, which can be better reflect the early PCB exposure in the human body (Artacho-Cordon et al. 2015). Therefore, breast adipose PCB concentrations might be more accurate to explain the association between breast cancer and PCB exposure than blood/serum PCB concentrations. In fact, it is almost impossible to get breast adipose tissues from healthy woman as controls well matched with age, residence, and collection time. In addition, controls from breast adipose tissues of benign breast disease are not ideal for on the same causal pathway as breast cancer cases (Hurley et al. 2011). Thus, it is important to explore the association between breast adipose PCB exposure in breast cancer patients and breast cancer development, which can provide clues for exploring the contribution of PCBs to human breast cancer.

Epidemiological studies on PCBs and breast cancer in China are few, and two case–control studies consistently showed that increased breast cancer risk was associated with serum PCB exposure (ORs were above 2 for PCB-118, PCB-138, PCB-153, and PCB-180 and total PCBs in one study, and OR was above 7 for PCB-52 in another study) (Ye 2009; Zhang et al. 2013). Information is lacking on PCB exposure in breast adipose tissue of women in China, and there is no study concerning the association of PCB exposure in human breast adipose tissue and breast cancer stage up to now. Our study aimed to investigate PCB exposure among Chaoshan women in China and evaluate the associations between PCB exposure in breast adipose tissue and breast cancer development.

Materials and methods

Subjects

The Chaoshan area consists of Shantou (Swatow), Chaozhou (Teochew), and Jieyang city and is located in the southeast coastal area of China. People here have a high frequency of fish and seafood consumption in their daily diet. Except for common habits of coastal people, they especially love drinking tea, which is a part of their daily lifestyle. Chaoshan population has a unique dialect and maintains their own traditional culture and customs, which made their dietary style and lifestyle distinctive in China.

In this study, we recruited 230 women older than 25 who were undergoing surgery for breast lumps at two hospitals serving the Chaoshan population: Cancer Hospital of Shantou University Medical College and The First Affiliated Hospital of Shantou University Medical College, from January 2014 to April 2015. We excluded women with any preceding cancer diagnoses and those who had received radiotherapy. A total of 223 women were eligible (mean age 51.69 ± 10.08 years [range 25 to 83]). Approximately 2 g adipose tissue was obtained from the mammary gland during surgery (14 cases of benign histological changes, 14 carcinoma in situ, and 195 malignant breast cancer). All participants gave their informed written consent after receiving detailed explanations of the study and potential consequences prior to enrollment, and a medical release form was obtained for permit to medical records and pathology reports. This study was performed with the approval of the Human Ethical Committee of Shantou University Medical College.

Epidemiologic data collection

Demography information and essential clinical data of patients were acquired from medical records. Demographic characteristics included age, height, weight, marriage, type of job, residential history, and lifestyle. Reproductive and clinical characteristics were also collected, including menarche age, history of menstrual disorders, menopause status, parity, history of lactation, blood type, preoperative chemotherapy, clinical stage, tumor–node–metastasis (TNM) classification, and history of breast disease as well as familial breast cancer. We extracted the information on pathological diagnosis of breast lumps from pathological reports, including malignant breast cancer, carcinoma in situ, and benign histological changes. Meanwhile, hormone receptor expression was also extracted, including estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2), and progesterone receptor (PR).

The TNM system was classified according to the size of the primary tumor (T), the lesion of regional lymph nodes (N), and distant metastases (M), established by the American Joint Committee on Cancer and the International Union for Cancer Control (Edge and Compton 2010). ER, PR, and HER2 expression in tumor was determined by immunohistochemical assay. ER and PR expression classification was coded as receptor negative with <10% of the cells stained for the receptor and receptor positive by the following criteria: 1+ (10~25%), 2+ (26~50%), 3+ (51~75%), and 4+ (76~100%). HER2 expression codes were based on the extent and the proportion of membranous staining in tumor cells: negative, ≤10% cells with no staining or weak and partial membranous staining; 1+, >10% cells with weak and partial membranous staining; 2+, >10% cells with moderate circumferential staining or ≤10% cells with strong circumferential membranous staining; and 3+, >10% cells with strong circumferential membranous staining.

Collection of samples

The collection of samples was previously described (Hernandez et al. 2009). Approximately 2 g breast adipose tissue was obtained from the subjects who were undergoing breast surgery, placed in hexane-washed polyethylene tubes, then frozen immediately, and stored at −80 °C, marking identity and disease classification.

Instrument analysis

Standards and solvents

The standard mixture (Mix 3, 100 μg/ml in cyclohexane) and individual PCB-198 (100 μg/ml in cyclohexane) were purchased from the Dr. Ehrenstorfer-Schafers Laboratories (Germany). The standard mixture containing the most familiar seven PCB congeners (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) in the environment is in extensive commercial use and well studied in experimental and epidemiological studies. The solvents used were *n*-

hexane, dichloromethane, and acetone (all pesticide grade; TEDIA); other chemicals were sulfuric acid (99.9%; Sigma-Aldrich) and anhydrous sodium sulfate (analysis grade; Tianjin, China), which were stored in drying vessels after baking at 300 °C for 18 h.

Sample processing and extraction

All experimental glassware used for PCB determination was washed with detergent in an ultrasonic cleaner for 30 min, scrubbed thoroughly with brushes, rinsed with ultrapure water three times, and then dried at 300 °C for 24 h in an oven. Then, the glassware was rinsed with acetone and *n*-hexane separately and dried by airing in a chemical hood before use. The procedure for specimen preparation, extraction, and purification of PCBs was as described (Covaci et al. 2008; Hernandez et al. 2009; Wu et al. 2011) with minor modification. Briefly, a procedural blank was run for every batch of 12 samples to verify cross-contamination. An amount of 0.5 g of tissue sample was spiked with surrogate standard PCB-198 as a sample recovery indicator and then homogenized with 3.0 g anhydrous sodium sulfate in agate mortar, and lipids were quantitatively extracted three times with 5 ml *n*-hexane each time, with shaking in a vortex. After filtration with microporous membrane, the combined extract was preconcentrated under a gentle nitrogen stream at 40 °C, and the final residue was used for gravimetrical lipid determination.

Sample purification

Extracted lipids were redissolved with 5 ml *n*-hexane and washed with concentrated H_2SO_4 three times. The washed lipids were shaken in a vortex for several seconds and then centrifuged for 6 min at 3000 rpm to separate the organic phase. The separated H_2SO_4 was extracted with *n*-hexane three times. The combined *n*-hexane extracts (approximately 20 ml) were reduced to 3 ml under a gentle nitrogen stream at 40 °C and loaded immediately onto a Florisil SPE cartridge to remove coextracted lipids and impurities (500 mg, 3 ml; CNW, Germany), which had been activated by eluting with 5 ml *n*-hexane and rested on a manifold vacuum station (Supelco, USA), and eluted with a mixture of 10 ml *n*-hexane/dichloromethane/acetone (3:1:1 *v*/*v*/*v*). The eluate was preconcentrated to dryness under gentle nitrogen stream at 40 °C, then redissolved in 50 μl *n*-hexane, and transferred to glass inserts in gas chromatography (GC) vials before GC–mass spectrometry (GC–MS) analysis.

Measurement and quantification

Samples were analyzed by an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, USA), with electron impact ionization used in the selected ion monitoring mode. PCBs were determined by auto-splitless injection (1 μl) with an Agilent GC automatic sampler (7683B) onto an Agilent 19091s-433 capillary column (30 m × 0.25-mm i.d., 0.25-μm film thickness), with helium (1.0 ml min^{-1}) used as the carrier gas. The column oven temperature was programmed from 110 °C (initial time, 0.5 min) to 200 °C at a rate of 18 °C/min, held for 1 min, then ramped from 200 to 280 °C at a rate of 8 °C/min, and held for 0.5 min. The total GC program was 17 min. The ion source, quadrupole, and interface temperatures were set to 230, 150, and 280 °C, respectively. The molecular and M-2 fragment ions were monitored as the target and confirmation ions. The target ion was used for quantification. Retention time was used to define the determined seven PCB congeners according to the retention time of PCB standards. Peak area was used to quantify PCB concentrations by the calibration curve of PCB standards.

Quality assurance and quality control

For every sequence, 13 samples were analyzed including a solvent blank, a procedural blank, and 11 samples. The procedural blank was used to monitor that the sample preparation processes were free of contamination. The solvent blank was added to ensure that the analysis process was not contaminated. The limits of detection (LOD), defined as three times the signal-to-noise ratio (S/N), were 0.09–0.32 ng g^{-1} for PCB. The recoveries of surrogate standard PCB-198 standard spiked in samples (n = 8) were from 67.2 to 93.8%, and the relative standard deviation was <12%. The precision of instrument, measured by detecting standards in the same day (n = 8) and consecutive 6 days (n = 8) and expressed as relative standard deviation (n = 11), at 5 ng g^{-1} levels, was below than 10% (indicating the good repeatability of the proposed method). Multilevel (five-point) calibration curves were constructed for quantification, with good to excellent linearity ($r^2 > 0.995$) achieved.

Statistical analysis

Statistical analyses involved use of SPSS 19.0 (SPSS Inc., Chicago, IL, USA). All statistical analyses were two tailed with $P < 0.05$ considered statistically significant. Non-detectable concentrations were coded as the value of the LOD divided by the square root of 2 (Holmes et al. 2014). We defined ∑PCB as the adipose sum of the concentrations of the seven congeners. Continuous variables are described with mean ± SD and non-normally distributed variables with median (interquartile range [IQR], *P*25–*P*75). Because PCB concentrations in adipose tissue were not distributed normally, Mann–Whitney *U* test and Kruskal–Wallis *H* test were used for comparing groups. *P* values were calculated by chi-square

test for categorical data. Spearman rank correlation analysis was used for correlation analysis.

Results

General characteristics of subjects

A total of 223 patients (mean age 51.69 ± 10.08 years) included 195 patients with invasive breast cancer, 14 with carcinoma in situ, and 14 with benign breast disease (Table 1). The average weight is a little overweight (mean BMI 24.03 ± 3.02). Of the patients, 59.2% resided in Shantou and 51.6% were peasant workers. Most (71.9%) had late menarche age (>14 years old); only 4.5% had early menarche age (≤12 years old). Overall, 56.9% were postmenopausal, 34.5% were housewives, 97.4% were parous, and 40.6% had more than three children. Natural breastfeeding (92.2%) was widely accepted, and 60.6% had a long lactation (≥12 months).

Table 1 General demographic characteristics of the participants (n = 223)

Characteristics	N	Percentage (%)
Age (mean ± SD, years)	51.69 ± 10.08	
BMI (mean ± SD, kg/m^2), n = 194	24.03 ± 3.02	
Type of disease		
Benign breast disease	14	6.3
Carcinoma in situ	14	6.3
Invasive breast cancer	195	87.4
Residence		
Jieyang	37	16.6
Chaozhou	42	18.8
Shantou	132	59.2
Other area	12	5.4
Type of job		
Peasant workers	115	51.6
White-collar employee	31	13.9
Housewives	77	34.5
Menarche age (mean ± SD, years), n = 199	15.20 ± 2.93	
≦12	9	4.5
12–14	47	23.6
>14	143	71.9
Menstrual disorders, n = 203		
Yes	13	6.4
No	190	93.6
Menopausal status, n = 218		
Postmenopausal	124	56.9
Premenopausal	94	43.1
Parity no., n = 190		
0	5	2.6
1–2	108	56.8
≥3	77	40.6
Ever breastfed, n = 193		
Yes	178	92.2
No	15	7.8
Lifetime duration of lactation (months), n = 137		
0	15	10.9
1–5	10	7.3
6–11	29	21.2
≥12	83	60.6

The distribution of PCB levels in adipose tissues and covariations among analytes

A total of seven PCB congeners were identified in adipose samples. The percentage of samples exceeding the limit of detection (% >LOD) was PCB-118 > PCB-153 > PCB-138 > PCB-180 > PCB-101 > PCB-52 > PCB-28 (Table 2). The predominant PCB congener was PCB-153, with the highest median mass concentration and median molar concentration (23.33 ng/g lipid and 64.63 pmol/g lipid, respectively), followed by PCB-138, PCB-180, PCB-118, PCB-101, PCB-52, and PCB-28 in molar concentration.

We found a significant correlation between ∑PCB concentration and that of each PCB congener (Supplementary tables, Table S1). Moreover, a significant positive linear association was found between each pair of PCB congeners except PCB-138 and PCB-52 (r = 0.048, P = 0.478). The strength of the correlation was highest for PCB-153 and PCB-138 (r = 0.889), followed by PCB-153 and PCB-180, PCB-153 and PCB-118, PCB-118, and PCB-52, and PCB-101 and PCB-28.

PCB level and general characteristics of subjects

On univariate analyses (Table 3), ∑PCB concentration increased by age with a borderline statistical significance (P = 0.061), and levels in 50–59- and ≥60-year-old groups are approximate. Compared with peasant workers, both white-collar employees and housewives showed relatively higher median PCB level (χ^2 = 14.024, P = 0.001). Also, as compared with the residents of Chaozhou, Jieyang, and other areas, those from Shantou had the highest PCB content (119.72 vs 85.29, 81.54, 106.19 ng/g lipid; χ^2 = 1.496, P = 0.009). But PCB level was not associated with BMI, blood type, and type of breast disease.

PCB level and breast carcinogenesis risk factors in breast cancer patients

There are 209 breast cancer patients. The breast carcinogenesis risk factors included age, history of benign breast disease, family history of breast cancer, menarche age, menopause status, parity, breastfeeding, menstrual disorders, age at menopause occurrence, postmenopausal BMI, and parity of women with ER/PR-positive tumor (Table 4). Median PCB concentration differed significantly by age groups (<55, 55–59, >59) and was highest in the 55–59-year-old group (χ^2 = 7.044, P = 0.03). The postmenopausal women had

Table 2 Total polychlorinated biphenyl (∑PCB) and PCB congener concentrations in breast adipose tissue in the participants (n = 223)

Compounds	N (%) >LOD	Median (ng/g lipid)	IQR (ng/g lipid)	Median (pmol g^{-1} lipid)	IQR (pmol g^{-1} lipid)
∑PCB	223 (100.0)	107.12	61.33–172.19	319.72	175.17–497.84
PCB-28	162 (72.6)	2.28	0.16–4.09	8.85	0.62–15.88
PCB-52	124 (55.6)	3.15	0.15–27.77	10.79	0.51–95.10
PCB-101	164 (73.5)	4.68	1.13–7.65	14.33	3.46–23.43
PCB-118	218 (97.8)	16.10	9.03–34.17	49.31	27.66–104.66
PCB-138	214 (96.0)	19.63	12.19–31.92	54.38	33.77–88.42
PCB-153	215 (96.4)	23.33	14.28–37.52	64.63	39.56–103.93
PCB-180	178 (79.8)	20.17	7.73–34.51	51.00	19.54–87.26

LOD limit of detection, *IQR* interquartile range (P25–P75)

higher median PCB level than premenopausal women (U = 4298, P = 0.038). Decreasing PCB level was associated with increasing parity in PR-positive women (χ^2 = 6.038, P = 0.049), but unsignificantly with parity in ER-positive women. However, median concentration of PCBs was lower but not significant for patients with a history of benign breast disease or family breast cancer than for other patients. In postmenopausal women, the PCB level was not related with BMI. As well, no association was found between PCB level and age at menarche, age at menopause, the number of parity, breastfeeding, or menstrual disorders.

PCB level and breast cancer clinical characteristics

Patients with preoperative chemotherapy were excluded because chemotherapy can inhibit the growth of the tumor. With increasing clinical stage, the median PCB level increased (P = 0.036). But the level did not regularly increase by

Table 3 Associations between general characteristics of subjects and ∑PCB level (ng/g lipid) (n = 223)

Characteristics	n (%)	∑PCB median (IQR)	χ^2	P value
Age (years)				
<40	20 (9.0)	54.04 (39.38–140.71)	7.364	0.061
40–49	76 (34.0)	103.96 (61.56–171.79)		
50–59	78 (35.0)	119.45 (64.51–201.88)		
≥60	49 (22.0)	119.67 (80.20–171.83)		
Type of disease				
Benign breast disease	14 (6.3)	97.07 (44.96–154.94)	1.173	0.556
Carcinoma in situ	14 (6.3)	128.52 (49.82–193.02)		
Invasive breast cancer	195 (87.4)	106.49 (61.54–176.16)		
BMI (kg/m^2), n = 194				
<18.5	6 (3.1)	129.85 (61.17–526.08)	0.335	0.846
18.5–23.9	96 (49.5)	101.46 (60.56–174.77)		
>23.9	92 (47.4)	105.30 (53.87–169.61)		
Type of job				
Peasant workers	115 (51.6)	91.60 (53.70–147.31)	14.024	0.001
White-collar employee	31 (13.9)	123.41 (81.10–188.95)		
Housewives	77 (34.5)	135.98 (83.05–234.52)		
Blood type				
A	72 (32.3)	115.89 (68.39–207.07)	3.616	0.164
B	72 (32.3)	100.04 (56.85–169.30)		
AB	11 (4.9)	61.33 (42.78–135.98)		
O	68 (30.5)	110.80 (61.39–170.11)		
Residence				
Jieyang	37 (16.6)	81.54 (54.90–120.87)	11.496	0.009
Chaozhou	42 (18.8)	85.29 (41.99–163.09)		
Shantou	132 (59.2)	119.72 (79.22–194.89)		
Other areas	12 (5.4)	106.19 (52.66–219.92)		

Table 4 Associations between breast carcinogenesis risk factors and ∑PCB level (ng/g lipid) in breast cancer patients (n = 209)

Characteristics	n (%)	Median (IQR)	Statistic	P value
Age				
≦55	135 (64.6)	98.89 (56.13–154.25)	χ^2 = 7.044	0.030
55–59	26 (12.4)	168.49 (83.15–230.43)		
>59	48 (23.0)	118.40 (79.74–172.47)		
History of benign breast disease[a]				
Yes	14 (6.7)	105.45 (52.34–173.4)	U = 1296	0.752
No	195 (93.3)	107.12 (61.60–184.53)		
Family history of breast cancer				
Yes	9 (4.3)	68.59 (57.05–165.53)	U = 753	0.408
No	200 (95.7)	107.72 (61.72–187.72)		
Age at menarche, n = 189				
≦12	9 (4.8)	68.59 (37.11–113.19)	χ^2 = 3.591	0.166
12–14	43 (22.8)	109.29 (53.7–266.38)		
>14	137 (72.4)	106.53 (64.72–172.05)		
Age at menopause, n = 108				
≦44	11 (10.2)	156.20 (100.70–277.64)	χ^2 = 3.120	0.373
44–49	30 (27.7)	126.56 (76.66–225.34)		
50–54	57 (52.8)	109.77 (61.78–192.55)		
>54	10 (9.3)	104.22 (86.51–194.88)		
Menstrual disorders, n = 192				
Yes	12 (6.3)	96.82 (52.50–169.37)	U = 1005	0.687
No	180 (93.7)	106.90 (60.56–175.56)		
Menopause status, n = 204				
Postmenopausal	117 (57.4)	127.27 (70.44–198.46)	U = 4298	0.038
Premenopausal	87 (42.6)	98.89 (50.20–160.94)		
Parity no., n = 183[b]				
0	5 (2.7)	99.71 (58.48–147.00)	χ^2 = 0.159	0.923
1–2	103 (56.3)	101.71 (59.94–172.19)		
≧3	75 (41.0)	106.98 (54.72–191.12)		
Ever breastfed, n = 187				
Yes	173 (92.5)	106.53 (55.56–172.89)	U = 1170	0.833
No	14 (7.5)	98.11 (78.78–138.49)		
Postmenopausal women's BMI, n = 102				
<18.5	3 (2.9)	205.22	χ^2 = 1.648	0.649
18.5–23.9	47 (46.1)	104.90 (75.99–200.77)		
24.0–26.9	29 (28.4)	146.43 (58.83–192.55)		
≧27.0	23 (22.6)	106.49 (51.75–173.74)		
Parity of women with ER+, n = 105				
0	4 (3.8)	108.45 (43.11–162.05)	χ^2 = 4.511	0.211
1	20 (19.1)	144.40 (103.69–161.86)		
2	44 (41.9)	106.01 (52.59–194.24)		
≧3	37 (35.2)	87.49 (52.53–149.31)		
Parity of women with PR+, n = 86				
0	0			
1	16 (18.6)	144.40 (114.41–200.51)	χ^2 = 6.038	0.049
2	37 (43.0)	133.47 (53.43–206.45)		
≧3	33 (38.4)	87.49 (52.53–133.37)		

[a] Time before breast cancer (years), mean (95% CI) = 12.07 (7.47, 16.67), and min–max = 1–20

[b] Parity no., median (IQR) = 2 (2–3), and min–max = 0–7

TNM classification and PR and HER2 expression (Table 5). With increasing PR and HER2 expression, the median PCB level changed non-linearly and unsignificantly. However, the association between ER expression and PCB level was statistically significant (χ^2 = 10.033, P = 0.04), but the median level did not regularly increase with increasing ER expression (negative, 83.68; 1+, 158.13; 2+, 100.21; 3+, 98.51; 4+, 128.11 ng/g lipid); similar results were found for levels of PCB-28, PCB-138, and PCB-153 (P = 0.025; P = 0.011; P = 0.026, respectively; data not shown).

Discussion

We investigated the concentration distribution of major PCBs in breast adipose tissue from women undergoing biopsy, lumpectomy, or mastectomy in Chaoshan, China. In order to explore the relationship between breast adipose PCB exposure and breast cancer development, we examined the associations between PCB exposure in breast adipose tissue and demographic characteristics, breast carcinogenesis risk factors, and clinical characteristics of tumor.

Table 5 Associations between clinical characteristics and ∑PCB level (ng/g lipid) in breast cancer patients (n = 174)

Characteristics	n (%)	∑PCB median (IQR) (ng/g lipid)	Statistic	P value
Clinical stages, n = 170				
0	11 (6.5)	95.27 (48.86–142.53)	χ^2 = 8.171	0.036
I	44 (25.9)	98.69 (58.99–158.13)		
II	83 (48.8)	110.62 (49.21–168.66)		
III + IV[a]	32 (18.8)	144.15 (65.79–227.84)		
T classification, n = 170				
T0	1 (0.6)	51.29	χ^2 = 5.262	0.261
Tis	11 (6.5)	95.27 (48.86–142.53)		
T1	62 (36.5)	114.15 (62.23–196.15)		
T2	90 (52.9)	100.21 (60.08–158.13)		
T3	6 (3.5)	85.55 (38.34–118.92)		
N classification, n = 170				
N0	88 (51.8)	106.56 (61.52–171.30)	χ^2 = 1.720	0.632
N1	60 (35.3)	100.12 (54.83–157.21)		
N2	13 (7.6)	146.42 (52.09–201.52)		
N3	9 (5.3)	76.48 (45.98–151.31)		
M classification, n = 170				
M0	167 (98.2)	104.11 (56.13–162.17)	U = 229.00	0.751
M1	3 (1.8)	60.08		
ER expression[b], n = 169				
Negative	51 (30.2)	83.68 (49.70–170.60)	χ^2 = 10.033	0.040[c]
1+	9 (5.3)	158.13 (85.45–213.80)		
2+	11 (6.5)	100.21 (66.28–196.15)		
3+	44 (26.0)	98.51 (49.05–146.98)		
4+	54 (32.0)	128.11 (79.58–210.45)		
PR expression[b], n = 169				
Negative	69 (40.8)	91.60 (51.65–174.13)	χ^2 = 1.844	0.764
1+	20 (11.8)	99.45 (54.05–203.39)		
2+	27 (16.0)	137.27 (60.30–196.15)		
3+	31 (18.4)	104.90 (51.75–151.30)		
4+	22 (13.0)	118.42 (73.32–157.12)		
HER2 expression[d], n = 169				
Negative	64 (37.8)	104.57 (53.65–160.81)	χ^2 = 2.180	0.536
1+	30 (17.8)	101.87 (53.39–169.07)		
2+	38 (22.5)	122.12 (64.72–220.79)		
3+	37 (21.9)	82.68 (57.31–171.35)		

[a] Because there are only three cases for the fourth stage of clinical stages, stage III and IV were combined

[b] Five expression groups of ER or PR are defined by the pathology classification method (negative, 1+, 2+, 3+, 4+)

[c] Levels of PCB-28, PCB-138, and PCB-153 also showed a significant difference by ER expression (P = 0.025; P = 0.011; P = 0.026. respectively), with the same change trend as ∑PCB level

[d] Four expression groups of HER2 are defined by the pathology classification method (negative, 1+, 2+, 3+)

PCB distribution and relationships with demographic characteristics

PCB exposure in women in Chaoshan, China, still remains high but is relatively lower than in European and American women (Aronson et al. 2000; Artacho-Cordon et al. 2015; Petreas et al. 2011). We analyzed seven PCB congeners—PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180—the most well-studied and plentiful PCBs in the environment. Because the toxic effect of PCBs in the human body is based on molecular units, we calculated not only mass concentration but also molar concentration. In agreement with many previous studies, PCB-153 had the highest mass concentration among those seven congeners (Hirai et al.

2005; Holmes et al. 2014; Muscat et al. 2003; Aronson et al. 2000). PCB-153 was also found to have the highest molar concentration in this study. Whether content was in mass or molar concentration, the order of median levels of PCB congeners was similar, except for PCB-138, with a slight higher median molar concentration than PCB-180 (in mass ng/g lipid: PCB-138 < PCB-180; in molar pmol/g lipid: PCB-138 > PCB-180). Therefore, mass concentration was used for the PCB level analysis, which also benefited the comparison with the PCB levels in other studies (Aronson et al. 2000, Petreas et al. 2011; Artacho-Cordon et al. 2015).

In this study, PCB-118 had the highest detection rate (97.8%). However, in a study of umbilical cord blood from healthy pregnant women in Guiyu in Shantou, PCB-138 was the most frequently detected (Wu et al. 2011). In theory, PCB-138 has a longer intrinsic human elimination half-life than PCB-118 (10.38 vs 9.3 years) (Ritter et al. 2011), so PCB-138 has a higher detection rate more likely when simultaneously exposed to the two congeners. The reasons for the different frequency of PCB-138 may be that the exposure mode of breast cancer women in our study differs from that of healthy women in the Shantou study (Wu et al. 2011). As levels of seven PCB congeners were strongly correlated with ∑PCB level (r from 0.415 to 0.837, all $P < 0.01$), ∑PCB level can represent the exposure of the seven individual PCB congeners. Therefore, in the following analysis and discussion, ∑PCB concentration was used to estimate the relationship between the investigated variables and PCB exposure.

Previous studies have shown that higher serum/adipose tissue PCB level correlated with older age (Costabeber and Emanuelli 2003; Bachelet et al. 2011), but this study showed a borderline significant association and an increasing trend among age groups <60 years but approximate levels between 50–59- and ≥60-year-old groups. In general, PCBs are accumulated and persist in the food chain and then enter the human body by ingestion because of their liposoluble characteristic and persistent properties. So, PCB body burden levels would increase with increasing age (Bachelet et al. 2011; Gasull et al. 2010).

PCB level changed with residence and occupation, with Shantou having the highest exposure among the Chaoshan cities and with housewives having higher PCB exposure than peasant workers and white-collar employee. This phenomenon is consistent with the characteristics of environmental pollutants. Whether BMI was positively associated with PCB level was ambiguous. In previous studies, Wolff et al. (2005) showed high PCB level in people with high BMI, whereas Bachelet et al. (2011) found high PCB level in those with low BMI. In this study, we found no difference in PCB levels by BMI. Similarly, PCB level was not found to be associated with breast disease classification (benign, carcinoma in situ, malignant).

Association between PCB levels in breast adipose tissue and breast cancer development

Breast cancer is attributed to many factors. Known risk factors mainly include hereditary, genetic, reproductive, hormonal, and environmental factors as well as lifestyle and preexisting breast conditions. Well-established reproductive risk factors for breast carcinogenesis include age, early menarche age, late age at menopause, postmenopausal obesity, no lactation, infertility, contraceptive abuse, and hormonal chemotherapy (Salehi et al. 2008); most are associated with the regulation of estrogen. However, increasing parity of women with ER/PR-positive tumor was reported to be a protective factor for breast cancer development (Ma et al. 2006). Environmental estrogen-like chemicals were suspected as potential risk factors for breast development by disturbing endogenous estrogen, such as PCBs, polybrominated diphenyl ethers, dioxins, perfluorooctane sulfonate, p,p′-dichlorodiphenyldichloroethane, and bisphenol A (He et al. 2016). PCBs are widespread and persistent in environment, which can interact with both sex steroid hormone and nerve thyroid hormone and disturb endocrine-dependent reproductive development (Buterin et al. 2006; Parent et al. 2011; Windham et al. 2015). In the present study, we chose some risk factors of breast carcinogenesis to assess their relationships with PCB exposure and explored the relationship between breast adipose tissue concentrations of PCBs and breast cancer development.

We observed that a considerable proportion of breast cancer patients (6.7%) had benign breast disease, with a mean time of 12.07 years before becoming breast cancer. Along with previous findings, patients with benign disease as controls in epidemiological case–control studies may not be ideal because women with proliferative benign breast lesions are on the same causal path as breast cancer patients and are at increased risk of breast cancer (Kabat et al. 2010). Use of controls with benign breast conditions may limit the ability to evaluate a risk for breast cancer. In addition, women whose a first-degree relative developed breast cancer were shown to have an increased risk of suffering from the cancer (Pharoah et al. 1997). Breastfeeding and increasing parity were considered as protective factors of breast cancer (Andrieu et al. 2006; Ma et al. 2006) but early menarche age, menstrual disorders, late age at menopause, and postmenopausal obesity as risk factors. In our study, no association was found between PCB level and those factors. Those results agree with some previous studies (Arrebola et al. 2015; Bachelet et al. 2011).

Apart from the previous results, there are interesting findings found in this study. The postmenopausal women had higher PCB level than premenopausal women. Late age at menopause was an established risk factor for breast cancer (Barnard et al. 2015; Tamimi et al. 2012; Yang et al. 2007),

but the association between PCB exposure with menopausal status and breast cancer risk was unclear. A study observed a lower risk of estrogen receptor-negative breast cancer with higher levels of PCB exposure in postmenopausal breast cancer women (Raaschou-Nielsen et al. 2005). However, another study showed that no association was found between PCB exposure and breast cancer in both premenopausal and postmenopausal women (Zhang et al. 2004). Given that age-specific incidence rates of female breast cancer are highest in the range of 55 to 59 years old in China, we divided the subjects into three groups: less than 55-, 55- to 59-, and more than 59-year-old group. The result showed that PCB level in the 55- to 59-year-old group was much higher than those in the other two groups significantly. The high-risk group with higher PCB level revealed that there may be some potential relationships between PCBs and breast cancer development. A meta-analysis showed that increasing parity can reduce breast cancer risk in women with ER-positive/PR-positive tumors (Ma et al. 2006). In our study, PCB level changed irregularly with increased parity in all subjects, but decreasing PCB level was associated with increasing parity in women with PR-positive tumors. So, higher PCB level might be related with higher risk of breast cancer development in women with PR-positive tumors. Further researches on the underlying mechanisms are needed.

As far as we know, there is a paucity of research investigating the relationship between breast cancer clinical stage and PCB exposure. In this study, a significant association was found between adipose tissue PCB level and breast cancer clinical stage, which suggested that adipose tissue PCB level was related with breast cancer development. Surely, the association may be limited by the cross-sectional study design, and further prospective studies are needed to confirm this association. We found no association between TNM classification and PCB level. PCB levels did not differ by PR and HER2 expression but did differ by ER expression ($P = 0.04$). With ER classification, PCB level showed a non-linear change (negative, 86.68; 1+, 158.13; 2+, 100.21; 3+, 98.51; 4+, 128.11 ng/g lipid), which was the same as a non-monotonic dose–response (NMDR) curve for endogenic E2 affecting the behavior and transcription of ER (Foster 2012). This non-linear change can be explained by the NMDR relationship of the endocrine effects in PCBs (Lagarde et al. 2015; Muto et al. 2002). An NMDR relationship of PCB level was also found with type II diabetes (i.e., a promoting effect in a certain range but inhibition below or above this range). We found that the levels of PCB-28, PCB-138, or PCB-153 also significantly differed by ER expression, with the same change trend as the ∑PCB level.

The biological toxicity of PCBs with 209 congeners is complicated and diverse. PCBs can induce transcripts of ER signaling pathways to activate ER response genes and stimulate cell proliferation in the MCF-7 cell line (Radice et al. 2008; Gjernes et al. 2012). However, lower-chlorinated PCB congeners can induce oxidative stress resulting in DNA damage and caspase-dependent apoptosis of human breast cancer cells. And below cytotoxic concentrations, lower-chlorinated PCB congeners still have toxic effects but only induce oxidative DNA lesions in breast cancer cells (Lin et al. 2009). According to structural, biological, and pharmacokinetic characteristics, PCBs were classified as three groups: estrogenic/neurotoxic, antiestrogenic, and immunotoxic (dioxin like) and enzyme-inducing [phenobarbital (PB)-type cytochrome P450] (Wolff et al. 1997). Due to the difference in biological toxicity of PCB congeners, different congeners may play different roles in the development of breast cancer and a certain congener with different levels may have different influence on breast cancer development by NMDR relationship (Hamers et al. 2011; Wolff and Toniolo 1995).

Strengths and limitations

Our study has some strengths. We measured PCB levels in breast adipose tissue instead of serum, which reflected the past and present human exposures and can better explain the relationship between PCB exposure and breast cancer. To our knowledge, our study is the first to investigate PCB exposure in breast adipose tissue in China as a developing country that lacks research in this field. Most epidemiological studies on PCBs and breast cancer were conducted in Europe and the USA, with few in developing countries (Shakeel et al. 2010). This study explored PCB exposure and pathological staging of breast cancer and by ER expression grouped into five levels, rather than negative and positive status that was showed in previous studies (Gatto et al. 2007; Hoyer et al. 2001; Zheng et al. 2000). We found that PCB level significantly differed by ER expression, with a non-linear change trend, which indicates the NMDR relationship of the endocrine effects of PCBs.

However, breast cancer susceptibility gene variant is also an important risk factor for breast carcinogenesis. Considering the relation of PCBs to those gene variants, more information will be provided to better explore breast adipose tissue PCB concentrations in relationship with breast cancer development.

Conclusions

In this research, we measured seven major PCB congeners in breast adipose tissues and explored the association between PCB exposure and breast cancer development. PCB exposure in women in Chaoshan, China, still remains at a high level. PCB-153 was the predominant PCB congener, followed by PCB-138, PCB-180, PCB-118, PCB-101, PCB-52, and PCB-28. Higher PCB level in breast adipose tissue was found to be

significantly associated with high breast cancer clinical stage, age at high incidence of breast cancer, postmenopausal women, and parity of women with PR-positive breast tumors. We found no association between TNM classification, PR or HER2 expression, and PCB level but found a NMDR relationship between ER expression and PCB level. Our findings suggested a potential association between PCB exposure and breast cancer development. However, this study was an observational research, and experimental studies in vitro and in vivo are needed to confirm these findings and explain the underlying mechanisms.

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Compliance with ethical standards All participants gave their informed written consent after receiving detailed explanations of the study and potential consequences prior to enrollment, and a medical release form was obtained for permit to medical records and pathology reports. This study was performed with the approval of the Human Ethical Committee of Shantou University Medical College.

Conflict of interest The authors declare that they have no conflicts of interest.

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