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Associations between internal exposure levels of persistent organic pollutants in adipose tissue and deep infiltrating endometriosis with or without concurrent ovarian endometrioma

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ABSTRACT

Endometriosis is a gynaecological disease characterized by the presence of ectopic endometrial tissue. Histologically, it appears as different sub-types, being peritoneal endometriosis, ovarian endometrioma (OvE) and deep infiltrating endometriosis (DIE), which are of major relevance due to their varying clinical presentations. A number of persistent organic pollutants (POPs) have been associated with the onset of endometriosis, yet the overall set of existing studies remains fairly divergent. In this preliminary case-control study we aimed to assess the potential associations between the internal exposure to POPs and the presence of DIE with or without concurrent OvE. Adipose tissue and serum samples were collected from surgically confirmed cases (n = 55) and controls (n = 44) enrolled during 2013 and 2015 in Pays de la Loire, France. Targeted pollutants (76 historical or more emerging POPs including dioxins, polychlorobiphenyls (PCB), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecanes (HBCDs) and organochlorine pesticides (OCPs) were quantified by chromatography coupled to mass spectrometry. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated from unconditional logistic regression adjusted for known confounding variables. The results showed significant associations between DIE and adipose tissue levels of 1.2.3.7.8 – PeCDD, OCDF, PCB 105, 114, 118 and 123, PBDE 183, PBB 153, and several OCPs including trans-nonachlor, cis-heptachlor epoxide, dieldrin, β-hexachlorocyclohexane and hexachlorobenzene. The largest associations were observed for OCDF followed by cis-heptachlor epoxide, exhibiting adjusted ORs (95% CI) of 5.42 (2.73–12.85) and 5.36 (2.44–14.84) per 1-SD increase, respectively. The stratified analysis comparing both disease sub-types suggested that adipose tissue exposure markers may be more associated with DIE concurrent with OvE, however these results need to be confirmed in a larger population.

1. Introduction

The evidence supporting the associations between exposure to endocrine disrupting chemicals (EDCs) and gynaecological diseases has been growing steadily for the last decade (Caserta et al., 2008; Gore et al., 2015). Endometriosis is a gynaecological disease characterized by the presence of endometrial glands and stroma outside the uterus and presents multiple non-specific symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia, dyschesia and sometimes infertility (Eskenazi et al., 2002; Giudice, 2010; Sampson, 1927). Histologically, it can be considered as three different sub-types, namely peritoneal endometriosis, ovarian endometrioma (OvE) and deep infiltrating endometriosis (DIE) (Nisolle and Donnez, 1997; Zondervan et al., 2002), which are supported by different genetic variants (Borghese et al., 2015). Invasive endometriosis in the form of OvE or DIE, while less frequent than the superficial subtype, warrant an independent evaluation from the superficial subtype regarding potential associations with POPs (Busacca and Vignali, 2003; von Theobald et al., 2016).

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Although the exact aetiology of endometriosis remains unclear, current dogma suggests that it is a multi-causal, estrogen-dependent disease, and responsive to the suppression of ovarian hormonal production which reduces the pain (Giudice, 2010). Among other EDCs, persistent organic pollutants (POPs) have been shown to bind the aryl hydrocarbon receptor (AhR) and to interfere with the signalling pathways involved in the adhesion and proliferation of endometrial cells (*e.g.* progesterone and/or estrogen pathways) (Aznaurova et al., 2014; Birnbaum and Cummings, 2002; Crain et al., 2008; Han and O'Malley, 2014). Experimental studies have shown consistent associations between exposure to dioxins and the onset of endometriosis in rodent and primate models (Cummings et al., 1999; Cummings et al., 1996; Johnson et al., 1997; Nayyar et al., 2007; Rier et al., 1993). A body of evidence in epidemiological studies also reports an association between POPs and endometriosis, mostly focusing on dioxins, polychlorinated biphenyls (PCBs), or organochlorine pesticides (OCPs) (Buck Louis et al., 2012; Heilier et al., 2005; Louis et al., 2005; Martinez-Zamora et al., 2015; Porpora et al., 2009; Upson et al., 2013). Unfortunately, brominated flame-retardants (BFRs) have been rarely investigated (Buck Louis et al., 2012; Hoffman et al., 2007). Despite these notable efforts, existing studies remain globally non–convergent and scarcely conclusive, likely due to methodological and population heterogeneity (Bruner-Tran and Osteen, 2010; Heilier et al., 2008; Smarr et al., 2016).

Considering a specific aetiopathology for the different endometriosis sub-types, we conducted a preliminary case-control study aiming to assess the potential associations between the internal exposure to POPs and the presence of DIE with or without concurrent OvE. In a precedent manuscript (Ploteau et al., 2016), we provided the methodological basis and the detailed results related to the characterization of the different POPs residual levels among participants. The purpose of the present work is then to explore the potential associations between these internal exposure levels and DIE with or without endometrioma.

2. Materials and methods

2.1. Study sample

Participants were enrolled as part of a case–control study performed in the Region Pays-de-Loire between 2013 and 2015 on a French population (Ploteau et al., 2016). Case individuals (n = 55) were 18 to 45 years old, with surgical diagnosis of DIE first based on clinical examination, of which 26 cases presented also OvE. For all cases, magnetic resonance imaging (MRI) and confirmatory surgery were then performed, as well as anatomo-pathological examination, with the objective of removing the lesions and confirming the diagnosis of DIE. All cases presented severe forms of endometriosis classified in stages III or IV according to the American Fertility Society reviewed-classification (AFSr) scale and often they were cases with rectosigmoid DIE exhibiting frozen pelvis with abundant adhesions. Control individuals (n = 44) were adult women of similar age and body mass index (BMI), consulting for other benign gynaecological conditions (tubal ligation, surgery for genital prolapse, ovarian cystectomy), without any clinical symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia or history of infertility, precluding the diagnosis of DIE in the absence of invasive exploration. The geographical origin and living area for all participants was similar favouring comparable underlying exposures. For both groups, the exclusion criteria were: history of cancer, suspected malignancy, autoimmune diseases, and any other chronic condition. Participants were identified during routine gynaecological examinations, being most of them sent by external contacts and referred for management of the disease after the diagnosis done. If they met the eligibility criteria, they were completely informed about the research protocol and invited to participate. Those women who accepted were then examined and interviewed, and surgery was underwent during the next two months. Detailed information was collected regarding diagnosis, anthropometric variables, and other factors potentially associated with exposure to POPs (Table 1), including age (years), BMI (kg/m^2), frequency and duration of breastfeeding (months). For case and control individuals, samples (2 m^3) of parietal fat (removed from the subcutaneous adipose tissue) and omental fat (adipose tissue removed from the abdominal cavity) were collected during surgery. A serum sample (20 mL) was also collected the day before surgery intended to perform an independent comparative analysis, for this reason number of samples are not coincident. This sampling was performed between July 2013 and September 2014. All samples were stored at − 80 °C until analysis. The protocol of this study was approved by the Bioethics Committee of the GNEDS (Groupe Nantais d'Ethique dans le Domaine de la Santé) and all patients signed an informed consent form.

Table 1
Study sample characteristics (mean ± standard deviation) from the adipose tissue and serum study including controls, cases of deep infiltrating endometriosis without ovarian endometrioma (DIE only) and DIE with ovarian endometrioma (DIE + OvE).

Adipose tissue study	Controls	Cases	
		DIE only	DIE + OvE
	(n = 44)	(n = 24)	(n = 25)
Age (years)	32.7 ± 6.4	32.7 ± 6.4	36.7 ± 4.7*
BMI (kg/m^2)	24.3 ± 4.6	23.5 ± 4.1	23.8 ± 5.2
Breast-feeding (months)	4.1 ± 14.9	2.4 ± 4.3	0.4 ± 1.2
Nulliparous women (n)	17	14	18
Parity (no birth)	1.7 ± 0.9	0.8 ± 1.0	0.5 ± 0.9
Lipid content (%)	66.1 ± 13.8	70.6 ± 9.8	68.2 ± 12.3
Serum study	Controls	Cases	
		DIE only	DIE + OvE
	(n = 26)	(n = 23)	(n = 25)
Age (years)	33.9 ± 5.9	32.8 ± 6.5	36.0 ± 5.4
BMI (kg/m^2)	24.7 ± 5.2	23.2 ± 3.9	23.5 ± 5.1
Breast-feeding (months)	6.1 ± 19.2	2.5 ± 4.4	0.4 ± 1.2
Nulliparous women (n)	6	14	16
Parity (no birth)	1.5 ± 1.1	0.7 ± 1.0	0.6 ± 0.9
Lipid content (%)	0.7 ± 0.1	0.5 ± 0.1***	0.5 ± 0.1***

* $p < 0.05$.
*** $p < 0.001$, Mann-Whitney-Wilcoxon Test for comparison between cases of endometriosis and controls.

2.2. Chemical analysis

The methodologies applied to isolate, detect, and quantify the targeted POPs including dioxins (n = 17 PCDD/F), polychlorobiphenyls (n = 12 dioxin-like + 6 non-dioxin-like PCB), polybromodiphenylethers (n = 8 PBDE), polybromobiphenyls (n = 3 PBB) and organochlorine pesticides (n = 30 OCPs) have been described elsewhere (Antignac et al., 2009; Antignac et al., 2006; Bichon et al., 2015; Cariou et al., 2005; Costera et al., 2006; Ploteau et al., 2016).

Briefly, ^{13}C-labeled congeners were added to each sample for quantification according to the isotopic dilution method. Fat and serum samples were first submitted to a high pressure and temperature extraction (ASE Dionex, Sunnyvale, CA, USA) or a liquid/liquid extraction with pentane, respectively. Resulting extracts were weighed to measure fat content (gravimetric method for fat, enzymatic determination for serum) and reconstituted in hexane for further sample clean up. Gel permeation chromatography (GPC) was used for isolating OCPs, while three purification steps using successively acid silica, Florisil®, and celite/carbon columns were applied for other targeted substances. PCDD/F, PCB, PBDE, PBB and OCP measurements were performed by gas chromatography (Agilent 7890A) coupled to high-resolution mass spectrometry (GC-HRMS) on double sector instruments (JEOL MS 700D and 800D) after electron impact ionization (70 eV), operating at 10000 resolutions (10% valley) and in the single ion monitoring (SIM) acquisition mode. HBCD isomers were quantified using liquid