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Metabolite of the pesticide DDT and incident type 2 diabetes in urban India

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ABSTRACT

Background: Previous epidemiological studies, largely conducted in high-income countries and cross-sectional, have suggested a relatively strong association between exposure to dichlorodiphenyldichloroethylene (DDE), a metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), and type 2 diabetes. DDT is widely used in India and the prevalence of type 2 diabetes there is increasing, but the association between these factors has not been explored to date.

Objective: The objective was to estimate the association of the p,p′ isomer of DDE with incident type 2 diabetes in India.

Methods: A nested case-control study was conducted in a representative prospective cohort of adults from two cities in India. Participants were enrolled in 2010–11 (n = 12,271) and followed for annual assessment of chronic diseases including type 2 diabetes. Baseline plasma samples from incident cases of diabetes (n = 193) and sex-city-matched controls (n = 323) were selected for analysis of p,p-DDE. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using conditional logistic regression.

Results: At baseline, cases had higher p,p-DDE concentrations: geometric mean (95% CI) 330 (273–399) ng/g lipid compared to 223 (189–262) ng/g lipid among controls. Delhi participants had higher p,p-DDE concentrations: 579 (521–643) ng/g lipid compared to 122 (102–145) ng/g lipid in Chennai. In unadjusted models, being in the highest versus lowest quartile of p,p-DDE was associated with a more than doubling of the odds of diabetes: unadjusted OR (95% CI), 2.30 (1.19, 4.43). However, this effect was no longer significant after adjustment for age: adjusted (95% CI), 0.97 (0.46, 2.06).

Discussion: Results suggest that levels of p,p′-DDE in Delhi are exceptionally high, but we did not observe a significant association between p,p-DDE and incident type 2 diabetes. As this is the first study to evaluate this association in India, more studies are needed to inform our understanding of the association in this context, including potential routes of exposure.

1. Introduction

The number of people with diabetes in India increased by nearly two-fold from 2007 to 2017 (International Diabetes Federation, 2017). Nationally, the prevalence of diabetes is high, especially in middle and old age, across all states and sociodemographic groups (Geldsetzer et al., 2018). Previous studies have documented the important role of aging and changes in lifestyle behaviours in this epidemic, but few have explored other potential risk factors such as endocrine disrupting chemicals (EDCs).

Dichlorodiphenyltrichloroethane (DDT) and its metabolite, dichlorodiphenyldichloroethylene (DDE), have been implicated as potent

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EDCs, and India is the only country that continues to produce DDT (Van Den Berg et al., 2017). The Endocrine Society's most recent Scientific Statement on EDCs concluded that the role of organochlorine pesticides such as DDT in the incidence of type 2 diabetes "currently represents the most solid association between a class of EDCs and the increase in the prevalence of type 2 diabetes" (Gore et al., 2015). A meta-analysis of 14 studies conducted through 2015 on the association between DDE and type 2 diabetes reported a summary odds ratio (OR) (95% confidence interval [CI]) of 1.95 (1.44, 2.66) (Evangelou et al., 2016), compared to, for example, a summary relative risk (95% CI) of 1.30 (1.20, 1.40) comparing high versus low intake of sugar sweetened beverages in 10 studies (Schwingshackl et al., 2017).

All of the 14 studies included in that meta-analysis were conducted in high-income countries, largely in North America and Europe, and most were cross-sectional (Evangelou et al., 2016). Given that type 2 diabetes is a chronic disease that develops over the course of several years (Martin et al., 1992), and that adipose compartment dynamics (i.e., weight gain or weight loss) may influence the levels of DDE in circulation (Wolff et al., 2007), prospective studies are critical for providing more conclusive evidence on this association. The objective of this study was to estimate the association of DDE measured in human plasma with incident type 2 diabetes using a nested case-control study design in a prospective cohort of adults from urban India.

2. Methods

2.1. Study population and sample collection

Centre for cArdiometabolic Risk Reduction in South-Asia (CARRS) is a prospective cohort study, established in 2010–11, when 12,271 men and non-pregnant women aged 20 years and above were enrolled in Delhi and Chennai, India (Nair et al., 2012). Fasting venous blood samples were collected for clinical biomarker assessment and biobanking. Specifically, 5 ml of venous blood was collected into a lavender-top Vacutainer tube containing EDTA and transported in coolers on ice packs to a facility where they were centrifuged for 15 min at 3500 rpm. Then 0.5 ml-aliquots of plasma were prepared in 1.0 ml cryovials and stored at −80 °C. For this ancillary study, plasma samples from baseline were pulled from storage and shipped on dry ice to the School of Environmental Sciences at Jawaharlal Nehru University for sample extraction.

After the baseline study visit, participants were contacted annually to collect information on self-reported diagnoses of chronic diseases including type 2 diabetes, and additional venous blood samples were collected for testing of clinical biomarkers at the second and fourth follow-up visits (2013 and 2015). Fasting plasma glucose (FPG) was measured using the hexokinase/kinetic method and glycated hemoglobin A1c (HbA1c) using high-performance liquid chromatography. Participants with non-missing data for FPG and HbA1c at the baseline and second follow-up visit (n = 7140) were eligible for inclusion in this ancillary study (Fig. 1).

This CARRS ancillary study was approved by the ethics review committees at Harvard University (IRB16–1092), Emory University (IRB00044159), and the Public Health Foundation of India (TRC-IEC-236/14). Written informed consent was obtained from all participants at baseline to use stored blood samples for "further laboratory tests for medical research."

2.2. Case and control definition

Incident type 2 diabetes was defined as having FPG < 126 mg/dl, HbA1c < 6.5%, and no self-reported physician-diagnosed diabetes at baseline (2010 − 11), and FPG ≥ 126 mg/dl, HbA1c ≥ 6.5%, or self-reported physician-diagnosed diabetes at one or more of the follow up visits (2012–15) (American Diabetes Association, 2017). Controls were defined as having all of the following at both baseline and throughout the follow-up period: FPG < 100 mg/dl, HbA1c < 5.7%, and no self-reported physician-diagnosed diabetes.

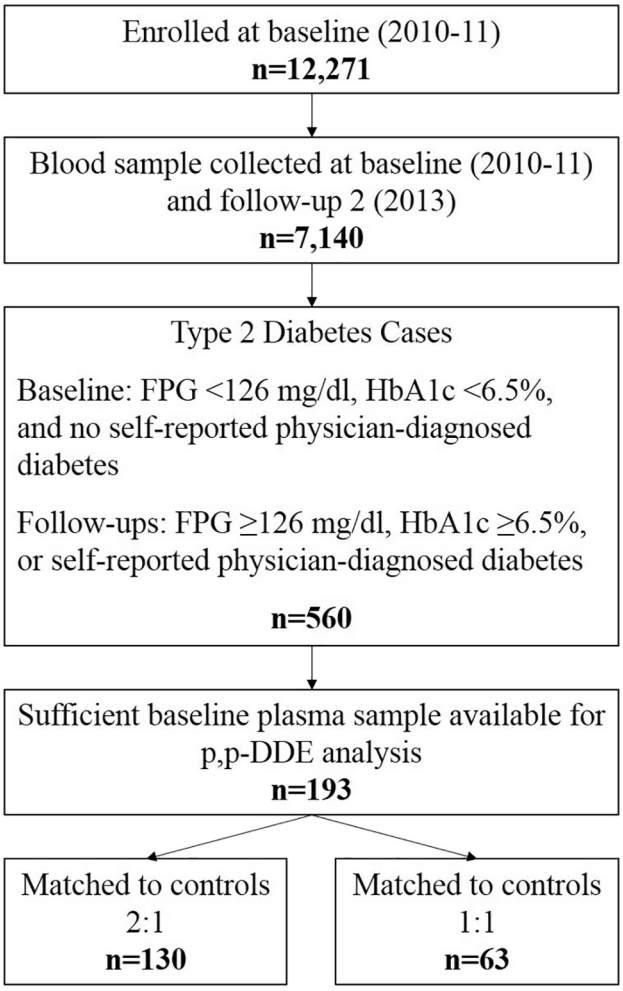

Fig. 1. Flow chart of participant inclusion in this nested case-control study conducted in a representative prospective cohort of adults.

A total of 193 participants met the case definition from Delhi and Chennai and had sufficient plasma sample (at least 500 µL) available for laboratory analysis of DDE. Controls were matched to cases 2:1 on sex (male or female) and city (Delhi or Chennai). Out of 104 total cases in Delhi, there were 30 male cases and 33 female cases that could not be matched to 2 controls and were matched 1:1. In sum, there were 130 case-control pairs matched 2:1 and 63 case-control pairs matched 1:1.

2.3. Biomarker analysis

Analysis of DDE focused on the p,p′ isomer (p,p-DDE). Plasma samples (17 participant samples), reagent and matrix blanks, and National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs 1958) were analysed concurrently in each run using a modification of existing methods (Marder et al., 2016; Gaspar et al., 2017). All samples (500 µL) were spiked with 1 ng of ring-labelled $^{13}C_{12}$-p,p-DDE as an internal standard. 2 ml of formic acid (50% v/v) was added to each sample to denature plasma proteins, then the samples were extracted twice with 5 ml of hexane. The hexane extracts were loaded onto the ISOLUTE Florisil cartridges topped with 0.5 g of anhydrous sodium sulfate and 1.8 g of acidified silica to remove residual biogenic materials, particularly lipids. The cartridges were

eluted with hexane:dichloromethane (19:1 v/v). Extracts were evaporated to dryness, reconstituted with 50 μL of nonane, and analysed using gas chromatography-tandem mass spectrometry.

The mass spectrometer was operated using electron ionisation technique. The target analytes (p,p′-DDE and its labelled analog) were monitored using multi-reaction monitoring mode. The concentration of the target analyte was determined from the peak ratio of analyte to labelled standard in the sample, by comparison to the standard calibration curve. The certified calibrants were obtained from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA). Quality assurance and quality control parameters included the use of NIST SRMs and blanks.

2.4. Covariate assessment

Sociodemographic characteristics including age, sex, educational attainment, occupational status, and monthly household income, as well as behavioural risk factors including tobacco and alcohol use, and dietary intake were assessed by a questionnaire developed based on questions used in previous studies (Nair et al., 2012; Iqbal et al., 2008). In particular, for dietary intake, a 26-item food propensity questionnaire adapted from the INTERHEART study (Iqbal et al., 2008) was used, which queried the frequency of consumption (never or less than once a month, per month, per week, or per day) over the past year. Thirteen food groups were evaluated: meat, poultry, seafood, eggs, dairy, vegetables, fruit, legumes, nuts, whole grains, refined grains, fried foods, and desserts.

Trained enumerators used standardised procedures to measure weight to the nearest 0.1 kg, height to the nearest 0.1 cm, and waist and hip circumferences to the nearest 0.1 cm based on the U.S. National Health and Nutrition Examination Survey protocol (Centers for Disease Control and Prevention, 1988). Body mass index (BMI) was calculated as weight (kg) divided by height-squared (m^2). BMI was categorised as normal weight (BMI $< 25\ kg/m^2$), overweight (BMI 25 - $< 30\ kg/m^2$), and obesity (BMI $\geq 30\ kg/m^2$). Urinary albumin was measured using an immunoturbidimetric assay and urinary and serum creatinine were measured using the kinetic Jaffe method with isotope dilution mass spectrometry traceable assays (Roche Diagnostics, Manheim, Germany). We defined chronic kidney disease as an albumin-to-creatinine ratio ≥ 30 mg/g or estimated glomerular filtration rate < 60 ml/min/$1.73m^2$ at baseline or the second or fourth follow-up visits (Anand et al., 2015; Eknoyan et al., 2013). The Chronic Kidney Disease Epidemiology Collaboration equation was used to estimate glomerular filtration rate (Levey et al., 2009).

Total cholesterol was measured in fasting serum samples using the cholesterol oxidase peroxidase timed-endpoint method, triglycerides were measured using the glycerol-3-phosphate oxidase enzymatic colorimetric method, and high-density lipoprotein (HDL) cholesterol was measured directly. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (Friedewald et al., 1972). Total serum lipid content was calculated using the Phillips formula (Phillips et al., 1989). Lipid-adjusted p,p-DDE concentrations (ng/g lipid) were used in the analysis.

2.5. Statistical analysis

Descriptive statistics were used to summarise the sociodemographic and clinical characteristics, behavioural risk factors (tobacco and alcohol use and dietary intake [13 food groups]), and p,p-DDE concentration of cases and controls at baseline. Given that the distribution of p,p-DDE was highly skewed (kurtosis in overall sample of 23.62 where a normally distributed variable would have a kurtosis near zero), we report geometric means (95% confidence intervals [CI]) throughout.

In order to determine the final confounder adjustment set (Rothman et al., 2008), we then analysed the association of these characteristics with quartiles of p,p-DDE among controls using descriptive statistics and Chi-square tests for binary and categorical variables or Kruskal-Wallis tests for continuous variables. We also evaluated the association between dietary intake and p,p-DDE among controls using multivariable linear regression, adjusting for city, sex, and age because we have previously reported that these three factors are important predictors of dietary intake in this cohort (Jaacks et al., 2016).

Given that the relationship between p,p-DDE and type 2 diabetes is likely non-monotonic (Lee, 2014), in order to estimate the association between p,p-DDE and incident type 2 diabetes we categorised p,p-DDE into quartiles according to the lipid-adjusted distribution among the controls. OR and 95% CI were estimated using conditional logistic regression (Hosmer and Lemeshow, 2000). Progressive degrees of multivariable adjustment were implemented: (1) crude model; (2) model adjusted for sociodemographic characteristics that were significantly associated with p,p-DDE among the controls; (3) model adjusted for all of the variables in (2) as well as waist circumference; and (4) model adjusted for all of the variables in (3) as well as FPG. This final adjustment was made because cases and controls differed in levels of FPG at baseline with cases having higher mean FPG (though still below the threshold for type 2 diabetes).

Sensitivity analyses were conducted that specified log-transformed p,p-DDE continuously as wet weight and two different approaches to accounting for blood lipid concentration: lipid-adjusted values (ng/g lipid) and wet weight adjusting for total lipid as a separate variable in multivariable models. We also formally evaluated the interaction between age and p,p-DDE in these models, and estimated models stratified by baseline BMI status (BMI $< 25\ kg/m^2$ versus $\geq 25\ kg/m^2$) and city (Chennai versus Delhi). In an additional series of sensitivity analyses, we used linear regression to estimate the effect of lipid-adjusted p,p-DDE (ng/g lipid) on the continuous outcomes of FPG and HbA1c at the second follow-up visit, stratified by case-control status, with progressive degrees of multivariable adjustment as described above. Finally, we compared p,p-DDE levels among cases with and without chronic kidney disease in an exploratory analysis.

Analyses were conducted using SAS v.9.4 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

Type 2 diabetes cases were older (mean 43.7 years versus 35.9 years in controls), were more likely to have a monthly household income > 20,000 INR (~$280 USD), were more likely to have a family history of diabetes, and had lower dietary intakes of refined grains compared to controls at baseline (Table 1). Cases also had higher levels of BMI, waist circumference, FPG, and HbA1c.

The geometric mean (95% CI) concentration of p,p-DDE among all 516 participants was 258.10 (227.71, 292.54) ng/g lipid with a maximum reported value of 6612.99 ng/g lipid. At baseline, cases had higher p,p-DDE concentrations: geometric mean (95% CI) of 329.96 (272.91, 398.94) ng/g lipid compared to 222.87 (189.25, 262.46) ng/g lipid among controls. Participants living in Delhi also had higher p,p-DDE concentrations: geometric mean (95% CI) of 578.96 (521.05, 643.31) ng/g lipid compared to 121.50 (101.55, 145.37) ng/g lipid among participants living in Chennai.

With respect to covariate associations, controls in the highest quartile of p,p-DDE were more likely to be from Delhi, male, working, to have a monthly household income > 20,000 INR, and to have ever used tobacco products (Table 2). Controls in the highest quartile of p,p-DDE also had higher waist circumferences and FPG at baseline. Adults in quartile 1 of p,p-DDE had higher dairy and fruit intakes compared to the referent (lowest) quartile of p,p-DDE, but no association was observed for either food group in higher quartiles of p,p-DDE (Supplementary Materials, pp. 1–3).

In unadjusted models, being in the highest versus lowest quartile of p,p-DDE was associated with a more than doubling of the odds of diabetes: unadjusted OR (95% CI), 2.30 (1.19, 4.43) (Table 3).

Table 1
Baseline characteristics of type 2 diabetes cases and controls living in urban India.

	Cases (n = 193)	Controls (n = 323)
City[a]		
Chennai, % (n)	46.1 (89)	55.1 (178)
Delhi, % (n)	53.9 (104)	44.9 (145)
Sex[a]		
Female, % (n)	56.5 (109)	57.3 (185)
Male, % (n)	43.5 (84)	42.7 (138)
Age (years)	43.7 ± 11.7	35.9 ± 10.4
Educational attainment		
No schooling up to primary school	21.2 (41)	17.0 (55)
High school up to secondary school	63.2 (122)	66.3 (214)
Graduate level or higher	15.5 (30)	16.7 (54)
Occupational status, % (n)		
Not working	57.0 (110)	52.3 (169)
Unskilled/semi-skilled	20.2 (39)	22.6 (73)
Trained/skilled or white collar	22.8 (44)	25.1 (81)
Monthly household income, % (n)		
≤10,000 INR (~$140 USD)	70.0 (135)	79.6 (257)
> 10,000 and ≤ 20,000 INR	18.1 (35)	10.5 (34)
> 20,000 INR (~$280 USD)	11.9 (23)	9.9 (32)
Family history of diabetes, % yes (n)	31.1 (60)	22.0 (71)
Ever use tobacco products, % yes (n)	25.4 (49)	21.1 (68)
Ever consume alcohol, % yes (n)	17.1 (33)	19.2 (62)
Vegetarian, % yes (n)[b]	22.8 (44)	18.3 (59)
Vegan, % yes (n)[b]	2.1 (4)	3.7 (12)
Meat intake (times/day)	0.13 ± 0.21	0.12 ± 0.25
Poultry intake (times/day)	0.12 ± 0.14	0.12 ± 0.14
Seafood intake (times/day)	0.15 ± 0.23	0.17 ± 0.22
Egg intake (times/day)	0.30 ± 0.37	0.31 ± 0.39
Dairy intake (times/day)	0.50 ± 0.52	0.49 ± 0.54
Vegetable intake (times/day)	1.65 ± 1.02	1.76 ± 1.84
Fruit intake (times/day)	0.57 ± 0.63	0.48 ± 0.52
Legume intake (times/day)	0.47 ± 0.40	0.52 ± 1.57
Nut intake (times/day)	0.13 ± 0.28	0.09 ± 0.22
Whole grain intake (times/day)	0.62 ± 0.90	0.57 ± 0.72
Refined grain intake (times/day)	1.38 ± 1.20	1.82 ± 1.99
Fried food intake (times/day)	0.18 ± 0.29	0.21 ± 0.34
Desserts intake (times/day)	0.34 ± 0.48	0.33 ± 0.44
p,p-DDE (ng/g lipid)	615.0 ± 827.8	454.9 ± 665.3
BMI (kg/m^2)	27.5 ± 5.0	23.4 ± 4.5
BMI status		
Normal weight (BMI < 25 kg/m^2)	29.5 (51)	66.4 (194)
Overweight (BMI 25 - < 30 kg/m^2)	41.6 (72)	25.3 (74)
Obesity (BMI ≥30 kg/m^2)	28.9 (50)	8.2 (24)
Waist circumference (cm)	90.4 ± 11.4	79.6 ± 11.4
Fasting blood glucose (mg/dl)	102.8 ± 10.7	89.4 ± 6.1
HbA1c (%)	6.0 ± 0.4	5.3 ± 0.3
Chronic kidney disease, % yes (n)[c]	4.2 (8)	2.5 (8)

Values are mean ± SD or % (n). Abbreviations: BMI, body mass index; DDE, dichlorodiphenyldichloroethylene.

[a] Matching factors.

[b] Vegetarian diets defined as eating meat, poultry, and seafood never or less than once a month. Vegan diets defined as eating meat, poultry, seafood, eggs, and dairy never or less than once a month.

[c] Defined as an albumin-to-creatinine ratio ≥30 mg/g or estimated glomerular filtration rate < 60 ml/min/1.73m^2 at baseline or the second or fourth follow-up visits.

However, this effect was no longer significant after adjustment for sociodemographic characteristics (age, occupational status, monthly household income, and tobacco use): adjusted OR (95% CI), 0.94 (0.51, 1.74). Further adjustment for waist circumference and FPG did not substantially change the effect estimate: adjusted OR (95% CI), 0.87 (0.30, 2.55). When each covariate was included in the model individually (Supplementary Materials, p. 5), age was identified as the strongest confounder: with adjustment for age alone, the OR (95% CI) was 0.97 (0.46, 2.06).

Results were consistent in sensitivity analyses modelling wet weight p,p-DDE (e.g., with no adjustment for total blood lipid content): unadjusted OR (95% CI), 1.34 (1.12, 1.60) and OR (95% CI) adjusted for the same set of sociodemographic characteristics (age, occupational status, monthly household income, and tobacco use), 1.06 (0.88, 1.26). Similarly, results were robust to modelling lipid-adjusted p,p-DDE concentrations continuously (log-transformed ng/g lipid): unadjusted OR (95% CI), 1.23 (1.03, 1.46) and OR (95% CI) adjusted for the same set of sociodemographic characteristics, 0.99 (0.83, 1.18). Adjusting for total blood lipid content as a separate variable did not substantially change the findings: OR (95% CI) adjusted for total blood lipid content, 1.16 (0.97, 1.39) and OR (95% CI) adjusted for total blood lipid content and the same set of sociodemographic characteristics, 0.97 (0.81, 1.17).

The interaction term for p,p-DDE and age was non-significant (p = 0.80 in unadjusted models and p = 0.71 in models additionally adjusted for occupational status, monthly household income, and tobacco use), and thus was not included in the final adjustment set. In models stratified by baseline BMI status, the OR (95% CI) adjusted for sociodemographic characteristics comparing the highest and lowest quartile of p,p-DDE was of greater magnitude among those with overweight/obesity (1.34 [0.23, 7.88]) than among those who were normal weight (0.59 [0.08, 4.34]). In models stratified by city, the unconditional logistic regression OR (95% CI) adjusted for sex and sociodemographic characteristics (age, occupational status, monthly household income, and tobacco use) for lipid-adjusted p,p-DDE concentrations (log-transformed ng/g lipid) was 0.97 (0.80, 1.17) in Chennai and 1.32 (0.89, 1.97) in Delhi. In linear regression models estimating the effect of baseline lipid-adjusted p,p-DDE (log-transformed ng/g lipid) on FPG and HbA1c at the second follow-up visit, no associations were observed in either controls or cases in both unadjusted and adjusted models (data not shown). Finally, in the exploratory analysis comparing p,p-DDE levels between cases with (n = 8) and without chronic kidney disease (n = 185), we found that 75.0% of cases with chronic kidney disease had p,p-DDE levels in the highest quartile compared to 37.8% of cases without chronic kidney disease.

4. Discussion

In this study of adults living in urban India, levels of p,p-DDE were high, especially in Delhi where the geometric mean concentration was 579 (95% CI: 521, 643) ng/g lipid compared to 268 (95% CI: 217, 332) ng/g lipid among adults the United States (most recent estimate, for 2003–2004) (Centers for Disease Control and Prevention, 2019). Male sex, employment, high monthly household income, and use of tobacco were predictive of higher plasma levels of p,p-DDE. Although the point estimate for the association of p,p-DDE with incident diabetes after adjustment for confounders, namely city and sex (matching factors), age, waist circumference (a proxy for central adiposity), and FPG, was compatible with a null finding, the 95% CI was wide, ranging from 0.30 to 2.55. As this is the first study to evaluate the association between plasma p,p-DDE concentration and incident type 2 diabetes in India, where the obesity transition is still in the early stages, more studies are needed to inform our understanding of the association in this context. These should include systematic studies of routes of exposure given the large difference in concentration observed between Delhi and Chennai.

While few previous studies have reported blood levels of p,p-DDE in India (Dhananjayan et al., 2012; Bhatnagar et al., 2004; Subramaniam and Solomon, 2006), the high levels of p,p-DDE found in Delhi in this study were not surprising considering that the National Vector Borne Disease Control Programme continues to recommend indoor residual spraying of households with DDT (the parent compound) for the control of malaria, visceral leishmaniasis, and other vector-borne diseases (Directorate General of Health Services, 2019). Since 2008, when China discontinued production, India has been the only country still producing DDT (Van Den Berg et al., 2017), and between 2000 and 2009, India accounted for 82% of global use of DDT for vector control, applying 3623 metric tons of DDT compared to 805 metric tons applied

Table 2
Association of sociodemographic and clinical characteristics with p,p-DDE concentration among controls (n = 323) living in urban India.

	Quartile of p,p-DDE				
	1 (n = 81)	2 (n = 80)	3 (n = 82)	4 (n = 80)	P-value*
City					< 0.0001
Chennai, % (n)	90.1 (73)	75.0 (60)	41.5 (34)	13.8 (11)	
Delhi, % (n)	9.9 (8)	25.0 (20)	58.5 (48)	86.3 (69)	
Sex					< 0.0001
Female, % (n)	77.8 (63)	65.0 (52)	54.9 (45)	31.3 (25)	
Male, % (n)	22.2 (18)	35.0 (28)	45.1 (37)	68.8 (55)	
Age (years)	32.6 ± 8.3	35.0 ± 9.2	36.6 ± 9.7	39.5 ± 12.7	0.0003
Educational attainment					0.19
No schooling up to primary school	17.3 (14)	21.3 (17)	13.4 (11)	16.3 (13)	
High school up to secondary school	72.8 (59)	62.5 (50)	70.7 (58)	58.8 (47)	
Graduate level or higher	9.9 (8)	16.3 (13)	15.9 (13)	25.0 (20)	
Occupational status, % (n)					0.002
Not working	66.7 (54)	56.3 (45)	52.4 (43)	33.8 (27)	
Unskilled/semi-skilled	21.0 (17)	20.0 (16)	18.3 (15)	31.3 (25)	
Trained/skilled or white collar	12.4 (10)	23.8 (19)	29.3 (24)	35.0 (28)	
Monthly household income, % (n)					< 0.0001
≤10,000 INR (~$140 USD)	91.4 (74)	78.8 (63)	78.1 (64)	70.0 (56)	
> 10,000 and ≤ 20,000 INR	8.6 (7)	17.5 (14)	6.1 (5)	10.0 (8)	
> 20,000 INR (~$280 USD)	0.0 (0)	3.8 (3)	15.9 (13)	20.0 (16)	
Family history of diabetes, % yes (n)	28.4 (23)	25.0 (20)	17.1 (14)	17.5 (14)	0.22
Ever use tobacco products, % yes (n)	12.4 (10)	16.3 (13)	24.4 (20)	31.3 (25)	0.02
Ever consume alcohol, % yes (n)	13.6 (11)	15.0 (12)	23.2 (19)	25.0 (20)	0.16
BMI (kg/m^2)	23.7 ± 4.5	24.3 ± 5.4	22.6 ± 4.0	23.1 ± 3.9	0.36
BMI status					0.03
Normal weight (BMI < 25 kg/m^2)	59.2 (42)	63.5 (47)	75.7 (56)	67.1 (49)	
Overweight (BMI 25 - < 30 kg/m^2)	33.8 (24)	20.3 (15)	18.9 (14)	28.8 (21)	
Obesity (BMI ≥30 kg/m^2)	7.0 (5)	16.2 (12)	5.4 (4)	4.1 (3)	
Waist circumference (cm)	76.8 ± 10.8	79.7 ± 12.0	79.4 ± 10.9	83.0 ± 11.1	0.02
Fasting blood glucose (mg/dl)	88.9 ± 5.1	87.3 ± 5.9	89.8 ± 6.9	91.6 ± 5.8	< 0.0001
HbA1c (%)	5.30 ± 0.23	5.28 ± 0.31	5.31 ± 0.27	5.19 ± 0.35	0.14

Values are mean ± SD or % (n). Abbreviations: BMI, body mass index; DDE, dichlorodiphenyldichloroethylene.
* p-Value from Chi-square test or Kruskal-Wallis.

across all of Africa (van den Berg, 2011). Previous studies of blood levels of p,p-DDE in India have been small convenience samples from Madurai (n = 22) (Subramaniam and Solomon, 2006), Ahmedabad (n = 18) (Bhatnagar et al., 2004), and Bangalore (n = 30) (Dhananjayan et al., 2012). The most recent (2012) and strongest (in terms of laboratory analytical technique) of these studies, in Bangalore, found that 80% of samples had detectable levels of p,p-DDE with a mean level of 5.67 μg/l (neither blood lipid-adjusted values nor geometric mean values were reported) (Dhananjayan et al., 2012). A recent study of South Asians living in the United Kingdom reported a median concentration of p,p-DDE > 8-fold higher than European whites, despite having lived outside South Asia for an average of 16 years: median of 535.87 ng/g lipid (range: 26.82 to 25,143.8 ng/g lipid) versus 61.26 ng/g lipid (range: 17.65 to 353.3 ng/g lipid) (Daniels et al., 2018). The median value reported in that study (535.87 ng/g lipid) is strikingly similar to the geometric mean value observed in Delhi in our study (579 ng/g lipid), but much higher than that in Chennai (geometric mean of 122 ng/g lipid).

In addition to the use of DDT for vector control, diet is likely to be another important exposure pathway. Several recent studies in northern India (in the state of Punjab, near to Delhi) have reported p,p-DDE is one of the most frequently detected residues in high-fat animal-based products such as milk and ghee (clarified butter) (Bedi et al., 2015; Bedi et al., 2016). This may at least partially explain the higher levels of p,p-DDE among adults living in Delhi versus Chennai as dairy and ghee consumption are higher in northern India versus southern India. Supporting this hypothesis, we observed higher dairy intake levels among participants in quartile 2 of p,p-DDE compared to those in quartile 1 (referent). More research is needed to understand specific routes of dietary exposure to p,p-DDE and other persistent organic pollutants in the context of India to inform recommendations for those wishing to reduce their exposure to these potential EDCs.

Table 3
Association of p,p-DDE concentration with type 2 diabetes among adults (n = 516) living in urban India.

	Quartile of p,p-DDE			
	1	2	3	4
Model 1: Unadjusted	1.00	1.13 (0.65, 1.99)	1.08 (0.59, 1.96)	2.30 (1.19, 4.43)
Model 2: Adjusted for sociodemographic characteristics[a]	1.00	0.93 (0.43, 1.99)	0.71 (0.36, 1.40)	0.94 (0.51, 1.74)
Model 3: Adjusted for Model 2 + waist circumference	1.00	0.69 (0.27, 1.76)	0.72 (0.33, 1.56)	0.82 (0.39, 1.72)
Model 4: Adjusted for Model 3 + FPG[b]	1.00	0.78 (0.20, 3.07)	1.06 (0.34, 3.34)	0.87 (0.30, 2.55)

Values are odds ratio (95% confidence interval) estimated from conditional logistic regression. Abbreviations: DDE, dichlorodiphenyldichloroethylene; FPG, fasting plasma glucose.
[a] Specifically, adjusted for age (years), occupational status (not working, unskilled/semi-skilled, or trained/skilled/white collar), monthly household income (≤10,000 INR, 10,000 – ≤20,000 INR, or > 20,000 INR), and ever use tobacco products (yes or no).
[b] Parameter estimates for all covariates provided in Supplementary Materials, p. 4.

Given that the half-life of p,p-DDE in blood is estimated to be 6 to 7 years (Ritter et al., 2009), the widespread application of DDT in India is likely to have long-term implications on human exposure levels. The persistence of p,p-DDE in blood was supported in our study by the strong positive association we observed between p,p-DDE and age. This is consistent with many previous studies documenting higher levels of p,p-DDE and other persistent organic pollutants among older populations (Zong et al., 2018). However, in contrast to some previous studies (Zong et al., 2018), we found that ever smoking was associated with higher p,p-DDE levels. We also found that employment and having a higher monthly household income were associated with higher concentrations of p,p-DDE, which could relate to greater intakes of high-fat foods, more frequent indoor residual spraying, or some combination of these and other factors in this setting.

Over the past 2 years, several studies have been published that evaluate the association between pesticides and diabetes among Asian populations. Similar to our study, a cross-sectional case-control study conducted in Phitsanulok Province, Thailand, largely among farmers (~75% of both physician-diagnosed diabetes cases and neighbor controls matched on sex and age), found no association between self-reported use of DDT (32 [7.1%] of cases and 48 [8.8%] of controls reported ever using DDT): the adjusted OR (95% CI) was 0.83 (0.50, 1.37) (Juntarawijit and Juntarawijit, 2018). This is similar to our adjusted OR (95% CI) of 0.87 (0.30, 2.55) comparing the highest and lowest quartiles of plasma p,p-DDE.

In contrast, the aforementioned cross-sectional case-control study among South Asians living in the United Kingdom found that type 2 diabetes cases (n = 24) were more likely to have plasma levels of p,p-DDE above the 50th percentile compared to controls matched on sex, age, proportion of Telugus, smoking status, and waist-hip ratio (n = 96): adjusted OR (95% CI), 7.00 (2.22, 22.06) (Daniels et al., 2018). This effect estimate is substantially higher than the summary OR (95% CI) reported in a recent meta-analysis of 14 studies that evaluated the association between p,p-DDE and diabetes: 1.95 (1.44, 2.66) (Evangelou et al., 2016). Interestingly, the adjusted OR (95% CI) for p,p-DDT, which is very similar in terms of chemical structure, was null in the UK study: 0.8 (0.33, 1.99) (Daniels et al., 2018) whereas the meta-analysis reported an OR (95% CI) of 1.79 (1.28, 2.49) for p,p-DDT (Evangelou et al., 2016).

Our findings of a null effect of p,p-DDE levels on incident type 2 diabetes in this population of urban Indian adults is inconsistent with decades of studies from animal and tissue culture models demonstrating biological plausibility (Howell III et al., 2014; Pavlikova et al., 2015; Kelce et al., 1995; Kuiper et al., 1998; La Merrill et al., 2014). Among controls we found that mean FPG levels were higher among those with the highest concentration of p,p-DDE: mean FPG of 91.6 mg/dl in the highest quartile of p,p-DDE compared to 88.9 mg/dl in the lowest quartile. Moreover, it has been posited that as the obesity transition progresses within populations – and with it, excess adiposity – the retention and toxicity of persistent organic pollutants such as DDT and DDE as relates to the risk of type 2 diabetes increases (Lee et al., 2006). The mean BMI among cases in this study was 27.5 kg/m^2, which is lower than that reported in previous prospective nested case-control studies in the United States: mean baseline BMI among cases of 32.6 kg/m^2 in the Nurses' Health Study II (Zong et al., 2018) and 29.6 kg/m^2 in Coronary Artery Risk Development in Young Adults (CARDIA) (Lee et al., 2010). The relatively low levels of excess adiposity in our sample may therefore be another explanation for the observed null effect.

Another explanation may relate to kidney function. A recent cross-sectional analysis of Mexican Americans in the National Health and Nutrition Examination Survey found that p,p′-DDE was not associated with diabetes without nephropathy, but was associated with diabetic nephropathy (Everett et al., 2017). Given that 95.9% of the incident type 2 diabetes cases in our sample did not have chronic kidney disease, our results are similar to that found for diabetes without nephropathy among Mexican Americans. Also consistent with that previous study, we found that the few type 2 diabetes cases in our sample that did have chronic kidney disease were more likely to have the highest levels of p,p-DDE. These results should be interpreted with caution given the small sample size (n = 8), but do suggest this may be an important direction for future research.

This study is not without limitations. As with any prospective epidemiological study, there may have been residual confounding in our estimates. In particular, we did not have information on breastfeeding history or history of weight change, which are thought to influence blood concentrations of persistent organic pollutants such as DDE and DDT (Zong et al., 2018; Zong et al., 2016; Milbrath et al., 2009). Our single-pollutant model is also subject to residual confounding by other persistent organic pollutants, particularly other organochlorine pesticide metabolites such as beta-hexachlorocyclohexane (parent compound: lindane), which are likely to be highly correlated with p,p-DDE. Moreover, we only had one measurement of p,p-DDE concentration, which may not reflect lifetime exposure even for persistent pollutants. We cannot rule out the possibility of a false negative or type II error, and we may have been underpowered to detect effect modification by age. If higher levels of adiposity are associated with greater toxicity of DDE, then one might predict larger effects among older participants given well-established changes in body composition that occur as individuals age (e.g., decreases in muscle mass [sarcopenia] and increases in body fat) (Borkan and Norris, 1977). The strengths of the study include its prospective design, relatively large sample size, and direct measurement of circulating p,p-DDE levels rather than relying on self-report.

In conclusion, results suggest that levels of p,p′-DDE in Delhi, India are exceptionally high, and much lower levels were observed in the southern, coastal city of Chennai. However, we did not observe a significant association between p,p-DDE and incident type 2 diabetes in this sample. As this is the first study to evaluate the association between plasma p,p-DDE concentration and incident type 2 diabetes in India, more studies are needed in order to make more definitive conclusions regarding the diabetogenic potential of this exposure in this context. Future research should continue to build laboratory capacity for environmental biomonitoring in India, specifically EDCs given the ever-growing diabetes epidemic.

Declaration of competing interest

The authors declare they have no actual or potential competing financial interests.

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LMJ, KMVN, DP, DBB, and SY conceptualised the study and obtained funding. IR, DM, and LMJ selected the plasma samples from storage and organised shipping to the laboratories in India and the United States. SY and LMJ ordered the analytical standards and reagents. SK and LMJ prepared the calibration solutions and quality control solutions. DBB and PP led hands-on training in the methodology in India. SK performed the sample preparation. HRG, SY, CJ, and PP performed the gas chromatography–mass spectrometry instrument analysis under the supervision of DBB. LMJ performed the data analysis and wrote the first draft of the paper. All authors provided feedback on the paper draft and approved the final version. LMJ is the guarantor of the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105089.

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