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Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil ☆, ☆☆

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ABSTRACT

Organochlorine (OC) pesticides are endocrine disruptors altering the thyroid hormonal system. The aim of this study is to investigate the relationship between exposure to OC pesticides and thyroid status in adults from a rural area in Rio de Janeiro, Brazil, heavily contaminated with OC pesticides. A cross-sectional study was carried out in 303 men and 305 women >14 years old. Concentrations of 19 OC pesticides and levels of free thyroxine (T4), total triiodothyronine (T3), thyroid-stimulating hormone (TSH), anti-thyroperoxidase (TPOAb) and anti-thyroglobulin (TgAg) antibodies were analyzed in serum samples. Associations between OC pesticides concentrations and values of biochemical thyroid parameters were determined using multivariate regression models stratified by gender. Prevalence of subclinical hyperthyroidism and the presence of TPOAb antibodies were higher than those described for euthyroid populations elsewhere. After adjusting for confounders, total T3 levels were associated with lower concentrations of endosulphan 2 in men and with higher alpha-chlordane, *p,p′*-dichlorodiphenyl-trichloroethane (DDT), endosulphan 2, and methoxychlor in women. Levels of free T4 showed inverse association with beta-hexachlorocyclohexane (HCH) and *p,p′*-DDT in men, and were positively associated with hexachlorobenzene (HCB), heptachlor, *o,p′*-DDT, and *p,p′*-DDT in women. TSH levels were associated with higher beta-HCH in men. A positive association was observed between exposure methoxychlor in males and presence of TPOAb, but no association with TPOAb was found in women. These results suggest that OC pesticides can affect the thyroid system through gender-specific mechanisms that may differ among compounds. Further detailed investigations and health monitoring should be warranted for this population.

1. Introduction

Organochlorine (OC) pesticides are highly lipophilic compounds with endocrine-disrupting activity. Given the structural similarity to thyroid hormones (thyroxine [T4] and triiodothyronine [T3]), OC pesticides may mimic action of several hormones, including thyroid function, and even modulate the mechanisms and interfere with their binding to hormonal receptors (Langer et al., 2007; Meeker et al., 2007; Zoeller, 2007), potentially leading to thyroid dysfunction (Alvarez-Pedrerol et al., 2008; Chevrier et al., 2008).

Thyroid hormones in humans are essential for metabolic regulation and for maintaining normal cardiovascular, reproductive, and nervous system functions. Thyroid-stimulating hormone (TSH), produced by the adeno-hypophysis, regulates the thyroid synthesis and the balance between T3 and T4 serum levels (Giacomini et al., 2006). Although the consequences of subclinical changes in thyroid function are currently under active discussion (Boelaert and Franklyn, 2005; Surks et al., 2004), all factors associated with any alteration in thyroid hormone levels may be of concern.

Animal studies have shown associations between elevated concentrations of OC pesticides such as dichlorodiphenyltrichloroethane (DDT), its main metabolite *p,p′*-dichlorodiphenyldichloroethane

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(DDE), and hexachlorobenzene (HCB), with lower thyroid hormone levels or higher TSH levels (Boas et al., 2006). Human data also indicate that chronic exposure to certain OC pesticides may interfere with the activity or metabolism of thyroid hormones and TSH (Langer, 2010). Thus, several epidemiological studies examined associations between OC pesticide exposure, among other OC compounds, and circulating levels of free T4, total T4, total T3, and TSH in cohorts characterized by low or moderate OC exposure (Bloom et al., 2003; Meeker et al., 2007; Pelletier et al., 2002; Sala et al., 2001; Schell et al., 2004, 2008). The observed findings in humans are, however, still inconsistent.

The potential of OC compounds to impair immune responses and to trigger autoimmune disease is also a matter of growing concern (Schell et al., 2009). In this regard, serum positivity of anti-thyroid antibodies (anti-thyroperoxidase [TPOAb] and anti-thyroglobulin [TgAb]) is a useful biomarker of future thyroid dysfunction, since they are indicative of thyroid injury or inflammation. They are also useful as a diagnostic tool for autoimmune diseases such as hypothyroidism (Cho et al., 2011), chronic thyroiditis (Langer et al., 2007), and thyroid cancer (Kim et al., 2010).

In a previous investigation carried out among children in a rural village in Southeast Brazil with a history of OC contamination from an abandoned pesticide production plant, increased total T3 levels was associated with OC pesticide serum concentrations (Freire et al., 2012). The present study aimed to examine the relationship between exposure to several OC pesticides and thyroid status among adult residents in the same area.

2. Material and methods

2.1. Exposure set

Cidade dos Meninos is a rural village located in the county of Duque de Caxias, State of Rio de Janeiro, Southeast Brazil. In the late 1940s, a factory was set up in this village for the production of hexachlorocyclohexane (HCH) and the formulation and storage of other OC pesticides, such as DDT and hexachlorobenzene (HCB), to be used by the Ministry of Health in vector control programs in Brazil. The factory was deactivated in 1955 and closed in 1961, but the remaining pesticide products were abandoned outdoors in the vicinity of the factory (Ministério da Saúde, 2003). Since then, local population has been chronically exposed to high levels of several OC pesticides found in soil, water, and local food. In particular, high levels of HCH isomers, DDT and other OC pesticides were found in soil, and ground and superficial water, as well as in locally produced food items such as eggs, milk, fruits and vegetables (Ministério da Saúde, 2003). Consequently, commercialization of local fruits and vegetables, eggs, poultry, meat and dairy products were forbidden by federal sanitary authorities. In 2008, results from a risk assessment revealed that ingestion of local food and dermal absorption through contact with contaminated soil were the main routes of exposure to OC pesticides in residents in Cidade dos Meninos (Asmus et al., 2008).

2.2. Study population and design

From November 2003 to March 2004, a population-based survey was conducted in Cidade dos Meninos to investigate the levels of serum OC pesticide amongst their residents. Population participating in this survey has been previously described (Freire et al., 2012). Briefly, a total of 381 families (1346 subjects) recorded from the 2003 official family census conducted by the Ministry of Health were contacted. From these, 359 families agreed to participate in the study providing a serum sample from at least one of their members. Participation rate was 96% of the 1346 initially identified subjects, but an electricity blackout resulted in loss of 26% of serum samples, limiting the chemical analysis to 995 (74%) residents. Among these, 787 were older than 14 years. Several biochemical parameters, including levels of free T4, total T3, TSH, and anti-thyroid antibodies, were also determined in serum samples of participants.

Four participants were excluded from the present investigation because they had extremely high serum concentrations of pesticides (*i.e.* levels of beta-HCH were higher than 1000 ng/ml). Additional 175 individuals had missing information on thyroid parameters or important covariates. Thus, a cross-sectional analysis was conducted on 608 adults (303 men and 305 women) with completed information on study variables. The study was approved by the Ethics Committee of the National School of Public Health (CEP/ENSP no. 1268/2004), Oswaldo Cruz Foundation (Fiocruz) in Rio de Janeiro, Brazil, and a signed informed consent was obtained from all participants in the study.

2.3. Data collection

2.3.1. Questionnaire

A validated questionnaire designed by the International Agency of Research on Cancer, including information about sociodemographic characteristics and lifestyle, was completed by study subjects through face-to-face interviews. Four trained interviewers collected all data immediately after blood sample collection and without previous knowledge on participants' exposure status. Variables derived from the questionnaire and used in the present analysis are: age (years), ethnicity (recorded as white or non-white), length of time residing in Cidade dos Meninos (years), period of life in Cidade dos Meninos (recorded as residents whose pregnancy occurred in Cidade dos Meninos and had lived there since birth; residents that moved to the study area during childhood [*i.e.* 1–14 years old]; or residents that moved during adulthood [> 14 years]), current alcohol use (any or none), cigarette smoking (recorder as never, ex-smoker, or current smoker), weight (kg), and height (cm). Women also provided information on breastfeeding history (any or none) and parity (as number of liveborn infants).

2.3.2. Laboratory analysis

Intravenous blood samples were collected under fasting conditions at the Primary Health Care Centre of Cidade dos Meninos. Blood samples were collected using a vacuntainer equipment and centrifuged at 2400*g* for 10 min to obtain sera, which were frozen at −20 °C until the analysis of pesticides and biochemical parameters. Concentration of OC pesticides was determined at the Laboratory of Toxicology of the Centre for Occupational and Human Ecology Health, Fiocruz. The analytical methodology, consisting of gas chromatography with electron-capture detection, has been previously described (Sarcinelli et al., 2003; Wolff et al., 1993). Target analytes were: HCH (alpha, beta and gamma isomers), HCB, chlordane (alpha and gamma isomers), *trans*-nonachlor, heptachlor, DDT metabolites (*p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDD), endosulfan 1 and 2, aldrin, endrin, dieldrin, methoxychlor, and mirex. The limits of detection were 0.02 ng/ml for HCH isomers, *o,p′*-DDT, *p,p′*-DDT, *p,p′*-DDD, endosulfan, endrin, methoxychlor, and mirex; 0.009 ng/ml for *p,p′*-DDE; 0.008 ppb for alpha and gamma chlordane, *trans*-nonachlor, heptachlor, aldrin, and dieldrin; and 0.004 ng/ml for HCB.

Levels of total T3, free T4, TSH, TPOAb, and TgAb were measured in serum samples by chemiluminiscence assay using ELISA kit (Alka Tecnologia®, São Paulo, Brazil) at the Clinical Pathology Laboratory in the Cancer Hospital of the National Institute of Cancer, Rio de Janeiro. Laboratory reference values ranged between 0.89 and 1.76 ng/dl for free T4, in the range 60–181 ng/dl for total T3, and 0.35–5.5 mU/l for TSH. Antibody reference values were up to 35 U/ml for TPOAb and up to 40 U/ml for TgAb. Assay detection limit was 10 U/ml for TPOAb and 20 U/ml for TgAb. Concentrations of total cholesterol and triglycerides were determined by colorimetric enzymatic methods at the same laboratory and expressed in milligrams per deciliter.

2.4. Statistical analysis

Any individual concentration of an OC pesticide that was below the limit of detection was substituted with the midpoint value between zero and the limit of detection of each compound. Pesticide concentrations were treated as continuous variables since all of them but methoxychlor (which was categorized into values below and above the detection limit) were detected in more than 60% of the participants. Free T4, total T3, and TSH levels were untransformed because they closely approximated normality. The presence of TPOAb and TgAb were defined as having antibody levels equal to or above the respective assay detection limit. Because TgAb was below the limit of detection in 93% of men and 89% of women, only the presence of TPOAb antibodies was examined in bivariate or multivariate analyses.

Spearman bivariate correlation analysis, *t*-test, and non-parametric test were conducted to examine gender-stratified relationships between characteristics of study population and serum levels of free T4, total T3, TSH, TPOAb, and OC pesticides. Linear regression coefficients with their 95% confidence intervals were computed to determine the association between serum concentrations of each OC pesticide and levels of thyroid hormones and TSH, while controlling for confounders and stratifying by gender. Odds ratios were calculated to assess the risk of TPOAb presence associated with each OC pesticide. Further multivariate analysis of data was conducted stratifying the study population according to three different windows of exposure, *i.e.* residents that had lived in Cidade dos Meninos since birth; those moving to the study area during childhood; or residents that moved during adulthood.

Confounders were chosen on the basis of bivariate associations ($p < 0.10$) with thyroid status biomarkers and/or OC pesticides, and of previous literature on risk factors for OC exposure (*i.e.*, BMI, parity, and breastfeeding). Models also included the wet-weight serum levels of pesticides and the serum lipid content (towards inclusion of cholesterol and triglycerides levels as covariates). A significance level of