The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand

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Abstract

It is now known that many organochlorines (OCs) act as endocrine disruptors, causing harmful effects on wildlife and humans. Several field and laboratory animal studies have reported that OCs cause adverse effects on thyroid hormone status. However, data regarding their effects on thyroid hormone status in humans are inconclusive. Because a developing fetus is especially sensitive to hormonal disruption by exposure to OCs, the adverse health effects on infants are of concern. The present study aimed to investigate the association between OC levels in maternal and cord serum, and the association between OC and thyroid hormone levels in cord serum. The study was performed with 39 mother–infant pairs from Mae Rim District of Chiang Mai Province, northern Thailand, who had normal delivery and full term gestation. Maternal blood was collected for measuring OCs and total lipids. Umbilical cord blood was collected for measuring OCs, total lipids, and thyroid hormones, including total thyroxine (TT4), free thyroxine (FT4), and thyroid stimulating hormone (TSH). 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (p,p′-DDE) had the highest level in all serum samples with a geometric mean of 1191 ng/g lipids in maternal serum and 742 ng/g lipids in cord serum. The second highest level was that for 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (p,p′-DDT), followed by 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (p,p′-DDD).

Levels of p,p′-DDE, p,p′-DDT, p,p′-DDD, and dieldrin in maternal serum were positively associated with levels in cord serum (r = 0.86, 0.77, 0.66, and 0.60, respectively; P < 0.001). The important findings were that cord serum TT4 levels were negatively associated with cord serum levels of p,p′-DDE (r = −0.37, P = 0.024), p,p′-DDT (r = −0.33, P = 0.048), and 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene (o,p′-DDE) (r = −0.76, P = 0.019). These results therefore suggest that exposure to DDT and its metabolites during fetal development may cause some effects on thyroid hormonal status in infants.

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Keywords: Organochlorines; Thyroid hormones; Infants; Endocrine disruption; Persistent organic pollutants; Prenatal exposure; DDT

1. Introduction

Organochlorines (OCs) have been extensively used for malaria vector control programs and farming purposes. Many OCs act as endocrine disruptors, causing harmful effects on wildlife and humans (Longnecker et al., 1997; Charlier and Plomteux, 2002). Recent studies in humans have reported the association of high-level exposure to OCs with infertilities, reproductive tract abnormalities, and breast cancer (Hoyer et al., 2000; Turusov et al., 2002; Toft et al., 2004; Windham et al., 2005). Available studies in infants have focused on the association with end points of adverse health effects such as low birth weight, preterm birth, growth retardation, and altered psychomotor and cognitive functions (Rylander et al., 2000;
Dormer and Plagemann, 2002; Siddiqui et al., 2003; Ribas-Fito et al., 2003a; Toft et al., 2004). In fact, the altered psychomotor and cognitive functions might be caused by alterations of thyroid hormone levels and metabolism (Porterfield and Hendrich, 1993). However, few studies investigated their effects on thyroid hormone levels in infants (Dewailly et al., 1993; Ribas-Fito et al., 2003b). In addition, field and laboratory animal studies have reported that exposure to OCs has an effect on thyroid hormone levels (Scollon et al., 2004; Verreault et al., 2004; Sormo et al., 2005).

In Mae Rim District, unusually high OCs, and dichlorodiphenyltrichloroethane (DDT) in particular, were detected in adult male plasma and breast milk (Stuetz et al., 2001; Asawasinsopon et al., 2006). DDT was applied for malaria vector control programs in this area from 1949 to 1989. It was also used for farming activities until it was officially banned in 1983, but most farmers had used it illegally despite its ban (Chareonviriyaphap et al., 2000; Stuetz et al., 2001). Pregnant women in this area might be exposed to these contaminants from environmental and occupational contributions, and their fetuses might be also exposed to these contaminants through placenta. Because exposure to OCs during fetus development may result in a permanent change of the endocrine system and neurodevelopment in infants, the adverse health effects on infants are of concern (Dorea et al., 2001; Sala et al., 2001a).

The present study aimed to investigate the association between OC levels in maternal and cord serum, and the association between OC and thyroid hormone levels in cord serum.

2. Methodology

2.1. Study population and study design

Between March 2003 and June 2004, all pregnant women living in Mae Rim District of Chiang Mai Province from northern Thailand for at least 5 years were invited to participate in the study. Because iodine deficiency during pregnancy has a strong impact on thyroid status in infants, pregnant women who had urinary iodine levels lower than 100 μg/l and who were diagnosed as hypothyroid were ruled out. Volunteers who were eligible and signed written consents were interviewed, using constructed questionnaires. Data on questionnaire consisted of personal data, lifestyle, pesticide use and exposure, and delivery history.

During delivery, 39 mother–infant pairs who had normal delivery and full term gestation (37 to 42 weeks of gestation) were study population. Ten milliliters of maternal blood was collected by venipuncture 2–5 h before delivery for measuring OCs and total lipids in serum. Twelve milliliters of umbilical cord blood was immediately collected when the umbilical cord was cut. OCs, total lipids, and thyroid hormones, including total thyroxine (TT4), free thyroxine (FT4), and thyroid stimulating hormone (TSH) were measured in cord serum. Anthropometric measures at birth, including weight, height, and head circumference, were obtained from hospital delivery records.

Characteristics of study population: A geometric mean age of the mothers was 23.8 years and the mean time of current residence was 12.6 years. The mean urinary iodine level was 244 μg/l (Min–Max 107–826 μg/l). Thirty-six mothers (92.3%) were farmers, of whom 11 (30.6%) had used DDT for farming purposes. The mean duration of DDT usage for farming was 3.8 years. Three mothers (7.7%) drank alcohol and 23 (58.9%) smoked cigarettes during pregnancy. Thirteen mothers (33.3%) were nulliparous, 9 (23.1%) were primiparous, and 17 (43.6%) were multiparous. Eighteen infants (46.2%) were males and 21 (53.8%) were females. The mean birth weight, height, and head circumference were 3007 g, 50 cm, and 34 cm, respectively. Only one infant (2.6%) had low birth weight (birth weight less than 2500 g).

2.2. Analysis of OCs

Maternal and cord serum were analysed for 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (p,p’-DDE), 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (p,p’-DDT), 1,1,2-dichloro-2,2-di(4-chlorophenyl)ethane (p,p’-DDE), 1,1,2-trichloro-2-(4-chlorophenyl)ethane (o,p’-DDE), 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (o,p’-DDT), heptachlor, heptachlor epoxide, dieldrin, hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), and gamma-hexachlorocyclohexane (γ-HCH). Standards of OCs were obtained from the US Environmental Protection Agency, NC, USA (p,p’-DDE, p,p’-DDT, and γ-HCH), laboratory of Dr. Ehrenstorfer, Augburg, Germany (o,p’-DDE o,p’-DDT, dieldrin, heptachlor, heptachlor epoxide, and aldrin), and Promochem Ltd., Wesel, Germany (p,p’-DDD, HCB, and β-HCH). All solvents for extraction were organic residue analytical grades (J.T. Baker, USA). The analytes were extracted by solid phase extraction using octadecyl (C18)-bonded silica cartridge (BondElut, Varian, USA). Extraction of these compounds from 2 ml serum samples was performed according to Prapamontol and Stevenson method (Prapamontol and Stevenson, 1991).

The isooctane extracts were analysed for OCs using gas chromatography–electron capture detection (GC–ECD). The GC analysis consisted of a Hewlett-Packard model 5890 Series II equipped with a 63Ni electron capture detector (ECD), an autosampler (HP 7673), a fused silica capillary column (Ultra 2:25 m×0.32 mm i.d. with 0.52 μm film thickness, J & W Scientific, USA), and a computerized data handling system (HP 3365 Series Chemstation). Temperature was 300 °C for the detection port and 200 °C for the injection port (splitless mode). Temperature programming of the oven was as follows: initial temperature 80 °C for 10 min, first ramp 30 °C/min to 190 °C, second ramp 4 °C/min to 250 °C, and the final temperature was held at 250 °C for 10 min. High purity helium (99.993%) was used as carrier gas and ultra high purity nitrogen (99.999%) was used as makeup gas.

Recoveries of individual OCs ranged from 70% for o,p’-DDT to 100% for HCB. Detection limit ranged from 0.01 ng/ml for o,p’-DDE to 0.24 ng/ml for p,p’-DDT. Intra-batch coefficient of variation (% CV) ranged from 4% for p,p’-DDT to 9% for heptachlor. Inter-batch % CV ranged from 6% for γ-HCH to 14% for p,p’-DDT. The intra- and inter-batches % CV for the most abundant congener, p,p’-DDE, were 5.9% and 5.8%, respectively. The determined mean levels for calculating
CVs ranged from 0.85 ng/ml for s-HCH to 22.81 ng/ml for \( p,p'\)-DDE.

2.3. Analysis of total lipids and hormones

Total maternal and cord lipids were measured using gravimetric methods (Blight and Dyer, 1959). Cord serum of thyroid hormones, including TT4, FT4, and TSH, were measured using radioimmunoassay (RIA-gnost\textsuperscript{®}, CIS bio International, France). Normal range of thyroid hormones in Thai infants reported by Ministry of Public Health, Thailand, was used. The infants who had lower TT4 and FT4 and higher TSH than the normal range for Thai infants were diagnosed as having primary congenital hypothyroidism. Intra-batch % CV was 6% for TT4, 2% for FT4, and 5% for TSH. The determined mean levels for total maternal and cord lipids were expressed on a lipid basis. SPSS version 11.0 was performed for data analysis. Descriptive statistic parameters, including geometric mean (GM), arithmetic mean (AR), median, standard deviation (S.D.), minimum (Min), maximum (Max), and percentile, were computed. The level of significance was set at a \( P\) value \(<0.05\) (2-tailed). The variables that did not approximate the normal distribution were natural logarithm transformed (ln) before parametric test. Pearson correlation coefficient (\( r\)) was used to investigate the association between OC levels in maternal and cord serum, and the association between OC and thyroid hormone levels in cord serum.

2.4. Statistical analysis

The levels of OCs below the detection limit were not reported. Because OCs are stored mainly in body fat, the levels were expressed on a lipid basis. SPSS version 11.0 was performed for data analysis. Descriptive statistic parameters, including geometric mean (GM), arithmetic mean (AR), median, standard deviation (S.D.), minimum (Min), maximum (Max), and percentile, were computed. The level of significance was set at a \( P\) value \(<0.05\) (2-tailed). The variables that did not approximate the normal distribution were natural logarithm transformed (ln) before parametric test. Pearson correlation coefficient (\( r\)) was used to investigate the association between OC levels in maternal and cord serum, and the association between OC and thyroid hormone levels in cord serum.

3. Results

3.1. OCs in maternal and cord serum

A total of 39 maternal and cord serum samples were analysed for 11 pesticides. The results of OC levels expressed on a lipid basis, and Pearson correlation coefficients are presented in Table 1. \( \beta\)-HCH was not detected in any serum samples; therefore, this compound was not included. \( p,p'\)-DDE had the highest level in all serum samples, with a geometric mean of 1191 ng/g lipids in maternal serum and 742 ng/g lipids in cord serum. The second highest level was that for \( p,p'\)-DDT, followed by \( p,p'\)-DDD.

Regarding the association between OC levels in maternal and cord serum, significantly high association was found for \( p,p'\)-DDE (\( r=0.86, P<0.001\)), \( p,p'\)-DDT (\( r=0.77, P<0.001\)), and \( p,p'\)-DDD (\( r=0.66, P<0.001\)), and significantly moderate association was found for dieldrin (\( r=0.60, P<0.001\)) (Table 1).

3.2. The association between OC and thyroid hormone levels in cord serum

Cord serum levels of thyroid hormones are presented in Table 2. The value at 95th percentile of cord serum TT4 and TSH was higher than the normal range. A positive association was found between TT4 and FT4, and significantly associated with TSH.

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Table 1

<table>
<thead>
<tr>
<th>OCs</th>
<th>Maternal No. of detected samples (%)</th>
<th>GM</th>
<th>AR</th>
<th>S.D.</th>
<th>Min</th>
<th>Max</th>
<th>Correlation coefficient in Maternal–ln Cor (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p')-DDE</td>
<td>Maternal 39 (100)</td>
<td>1191</td>
<td>1792</td>
<td>1579</td>
<td>58.3</td>
<td>7981</td>
<td>0.86***</td>
</tr>
<tr>
<td></td>
<td>Cord 39 (100)</td>
<td>742</td>
<td>1028</td>
<td>866</td>
<td>81.3</td>
<td>4265</td>
<td></td>
</tr>
<tr>
<td>( p,p')-DDT</td>
<td>Maternal 39 (100)</td>
<td>123</td>
<td>195</td>
<td>217</td>
<td>18.0</td>
<td>1067</td>
<td>0.77***</td>
</tr>
<tr>
<td></td>
<td>Cord 37 (94.9)</td>
<td>77.1</td>
<td>103</td>
<td>112</td>
<td>21.5</td>
<td>660</td>
<td></td>
</tr>
<tr>
<td>( p,p')-DDD</td>
<td>Maternal 39 (100)</td>
<td>104</td>
<td>139</td>
<td>115</td>
<td>16.8</td>
<td>527</td>
<td>0.66***</td>
</tr>
<tr>
<td></td>
<td>Cord 39 (100)</td>
<td>89.1</td>
<td>105</td>
<td>63.0</td>
<td>24.1</td>
<td>309</td>
<td></td>
</tr>
<tr>
<td>( o,o')-DDE</td>
<td>Maternal 8 (20.5)</td>
<td>21.6</td>
<td>24.8</td>
<td>11.2</td>
<td>5.8</td>
<td>37.1</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Cord 9 (23.1)</td>
<td>46.6</td>
<td>51.9</td>
<td>27.4</td>
<td>23.9</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>( o,o')-DDT</td>
<td>Maternal 19 (48.7)</td>
<td>21.5</td>
<td>23.3</td>
<td>21.1</td>
<td>7.0</td>
<td>100</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Cord 10 (25.6)</td>
<td>17.1</td>
<td>18.1</td>
<td>6.5</td>
<td>10.0</td>
<td>27.8</td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>Maternal 35 (89.7)</td>
<td>68.0</td>
<td>91.9</td>
<td>94.1</td>
<td>15.9</td>
<td>533</td>
<td>0.60***</td>
</tr>
<tr>
<td></td>
<td>Cord 37 (94.9)</td>
<td>94.9</td>
<td>120</td>
<td>116</td>
<td>32.4</td>
<td>639</td>
<td></td>
</tr>
<tr>
<td>Heptachlor</td>
<td>Maternal 32 (82.1)</td>
<td>20.8</td>
<td>27.2</td>
<td>22.6</td>
<td>3.8</td>
<td>111</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Cord 31 (79.5)</td>
<td>37.1</td>
<td>64.3</td>
<td>103</td>
<td>9.1</td>
<td>545</td>
<td></td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>Maternal 32 (82.1)</td>
<td>39.6</td>
<td>45.2</td>
<td>29.0</td>
<td>17.8</td>
<td>177</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Cord 29 (74.4)</td>
<td>38.8</td>
<td>45.9</td>
<td>27.3</td>
<td>15.0</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>Maternal 14 (35.9)</td>
<td>17.3</td>
<td>18.9</td>
<td>8.0</td>
<td>6.3</td>
<td>38.4</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Cord –</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>( γ)-HCH</td>
<td>Maternal 8 (20.5)</td>
<td>16.1</td>
<td>53.0</td>
<td>119</td>
<td>4.0</td>
<td>347</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Cord 2 (5.1)</td>
<td>29.1</td>
<td>34.2</td>
<td>25.4</td>
<td>16.3</td>
<td>52.2</td>
<td>a</td>
</tr>
</tbody>
</table>

*The statistic cannot be computed; ***\( p<0.001\).

---

Table 2

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>5th–95th Percentile</th>
<th>Normal range of Thai infants(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT4, ( \mu g/dl)</td>
<td>8.65</td>
<td>4.00</td>
<td>13.00</td>
<td>6.28–13.00</td>
<td>5.30–11.80</td>
</tr>
<tr>
<td>FT4, ( ng/dl)</td>
<td>1.05</td>
<td>0.50</td>
<td>1.60</td>
<td>0.80–1.51</td>
<td>0.80–1.60</td>
</tr>
<tr>
<td>TSH, ( mIU/l)</td>
<td>9.55</td>
<td>2.70</td>
<td>33.70</td>
<td>3.76–31.80</td>
<td>2.30–24.20</td>
</tr>
</tbody>
</table>

\(^a\) Normal range of Thai infants from Ministry of Public Health, Thailand.
OCs were detected in both maternal and cord serum, indicating that these chemicals have an efficient transplacental transfer (Kanja et al., 1992; Bjerregaard and Hansen, 2000; Waliszewski et al., 2001; Sala et al., 2001a; Butler et al., 2003). Rate of transplacental transfer of chemicals depends on characteristics of placental membrane and chemicals, placental blood flow, plasma protein binding of chemicals, and respective pHs of the maternal and fetal circulation (Pratt, 1990). Regarding the characteristics of chemicals, lipophilic and low molecular weight chemicals, including OCs, are fully capable of crossing the placental membrane by passive diffusion (Morgan, 1997). The transfer also depends on lipid solubility characteristics of individual chemicals, meaning that more lipid-soluble chemicals attain equilibrium between mother and fetus more rapidly (Klaassen and Rozman, 1996).

A high Pearson correlation coefficient between OC levels in maternal and cord serum indicates an equilibrium pattern between these body compartments during pregnancy (Dorea et al., 2001; Waliszewski et al., 2000). In the present study, a high correlation coefficient was found for $p,p'\text{-DDE}$, $p,p'\text{-DDT}$, and $p,p'\text{-DDD}$. It can be concluded that transplacental transfer of these three compounds reaches a balanced state between mother and fetus. A fetus has low cytochrome P450 enzyme-mediated biotransformation of OCs, resulting in a weak detoxification process (Waliszewski et al., 2001). Therefore, OCs are accumulated in body compartments of the fetus, subsequently causing adverse health effects.

### 4.2. The association between OC and thyroid hormone levels in cord serum

Several studies in humans have focused on effects of HCB, polychlorinated biphenyls (PCBs), and dioxins on thyroid hormone levels. HCB levels were negatively associated with TT$_4$ levels, but not associated with TSH and FT$_4$ levels in an adult population (Sala et al., 2001b). Prenatal exposure to HCB was not associated with TSH levels in infants (Ribas-Fito et al., 2003b). PCBs and dioxin levels in breast milk were negatively associated with T$_4$ and T$_3$ levels, and positively associated with TSH (Koopman-Esseboom et al., 1994; Nagayama et al., 1998). In other studies, PCBs and dioxins levels in breast milk and maternal serum were not associated with thyroid hormone levels, including T$_4$, T$_3$, and TSH (Fiolet et al., 1997; Longnecker et al., 2000; Steuerwald et al., 2000; Matsuura et al., 2001). However, so far the association between DDT and thyroid hormones in cord serum was scarcely reported.

Field and laboratory animal studies have reported that DDT has an effect on T$_4$ and T$_3$ levels (Scollon et al., 2004; Verreault et al., 2004; Sormo et al., 2005). DDT levels were negatively associated with plasma T$_4$ levels in male glaucous gulls, and plasma T$_3$ in gray seals (Verreault et al., 2004; Sormo et al., 2005). In laboratory animal studies, T$_4$ decreased in flown birds dosed with DDT and T$_3$ decreased in fasted birds dosed with DDT (Scollon et al., 2004). DDT has been shown to disrupt thyroid hormones by increasing peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes, resulting in an increased excretion of plasma thyroxine (Fregly, 1968; Bastomsky, 1974; Capen, 1994; Scollon et al., 2004). DDT does not bind to thyroid receptor, indicating that it is unlikely to disrupt the thyroid axis through a negative feedback mechanism (Cheek et al., 1999). Therefore, DDT is unlikely having an affect on TSH secretion.

In the present study, only cord serum TT$_4$ levels were negatively associated with cord serum levels of $p,p'\text{-DDE}$, $p,p'\text{-DDT}$, and $o,o'\text{-DDE}$. However, TT$_4$ levels of most subjects (92.3%) were within the normal range. It is possible that serum DDT levels might not be high enough to have an obvious effect on hormonal metabolism. Another possibility is that the association for $o,o'\text{-DDE}$ may be a chance finding due to multiple comparison, since $o,o'\text{-DDE}$ in the present study was detected in only 23.1% of the study population, and only 9 tests of association were therefore analysed.

In fact, humans are exposed to a variety of the OC contaminants and the levels of individual contaminants in the body might be rather closely interrelated. It is therefore difficult to assess which compounds have an effect on hormonal status (Hagmar, 2003). Our results therefore suggest that exposure to DDT and its metabolites during fetal development may cause some effects on thyroid hormonal status in infants. Despite the somewhat limited sample size, the sample comprises 73.6% of all mothers who gave a birth during March 2003–June 2004 in this area, and the results are quite compelling.

### 5. Conclusion

The results of the present study indicate that OCs have an efficient placental transfer, and prenatal exposure to DDT and its...
metabolites may cause some effects on thyroid hormone levels in infants. Thyroid hormones play an important role in brain and neurodevelopment of infants. Therefore, the small change of hormonal levels may cause irreversible changes during development and affect later functioning in adult life. These findings emphasize the need to further investigate the adverse effects on thyroid hormones, growth, and neurodevelopment in children exposed to high doses of DDT.

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