Breast cancer risk assessment and its correlation with the residual level of DDT in blood and tissue of people of Bihar, India.

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ABSTRACT
A study of breast cancer cases using hospital clinical data was conducted in the state of Bihar, India. It was found that in the past ten years breast cancer incidence increased cumulatively by 642%. In this investigation main emphasis has been given on breast cancer patients. Five districts (Khagaria, Muzaffarpur, Samastipur, Sitamarhi and Vaishal) in the state of Bihar were selected for residual DDT analysis in serum and tissue of a sample population. Pesticides were estimated by using High Pressure Liquid Chromatography (HPLC). Total DDT level in serum samples ranged from 5 ppb to 25 ppb and in the tissue samples it ranged from 900 ppb to 4300 ppb. Maximum accumulated DDT residue in tissue was recorded in the district of Samastipur. Highest accumulation of pp'-DDE was found in the both serum and tissue samples followed by pp-DDT, op-DDT, and pp'-DDD. Increasing concentration of DDT residues in tissues was found to be directly proportional to the effect on estrogen receptors in tissues. This study demonstrates that high accumulation of DDT or DDE and negative effects on estrogen receptor is strongly correlated to carcinogenicity in breast cancer patients.

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Materials and Methods

Breast Cancer Survey

Over the last 10 years a survey was carried out among the breast cancer patients visiting Mahavir Cancer Sansthan Hospital from the different districts of Bihar. A total of all cancer cases, breast cancer cases data were obtained from the Department of clinical pathology, Mahavir Cancer Sansthan, Patna, India. Finally, the obtained data were collected and categorized.

Chemicals

HPLC standard fine chemicals and solvents were purchased from Qualigens Chemicals, India. Pure (100%) pesticide standards DDT and its metabolites were supplied by Sigma Chemicals, India.

DDT and its metabolites measurements

Five districts of Bihar were selected for the present study, which has more prevalence of breast cancer. Blood and tissue samples were collected from twenty breast cancer cases of each district. Pesticide chemicals extraction from blood and tissue was carried out based on the method followed by Mathur et al., (2005), and Darko and Acquaah (2007) respectively. About 5 ml of blood was collected in red-top Vacutainers. The collected blood was allowed to clot at room temperature. After 30 min incubation blood sample was centrifuged at 3,000 rpm for 15 min. Serum was collected and frozen at -20°C. 1 ml of serum of was vigorously shaken with 5 ml of hexane in a cyclomixer. The hexane layer was concentrated to 200 ml under vacuum. Three gram of tissue sample was collected in glass vials. Tissue sample was homogenized using a Waring blender. The analyzed compounds were extracted from the sample with the use of hexane and acetonitrile. Finally the solvent was evaporated using under vacuum. Three replicates were maintained for each sample. The obtained extracts both from blood and tissue samples were subjected to High Pressure Liquid Chromatography (HPLC).

Histopathology

Histopathological changes were observed in the cancer tissues. Tissues were taken from the five breast cancer cases having DDT residues at various concentrations on the serum and tissue. Obtained tissues samples were processed as per the routine standard methods. The tissue sections were stained with Delafield’s hematoxylin, counter stain in eosin and mounted in DPX for light microscopy.

Statistical analysis

Levels of DDT and its metabolites residues were analyzed using one way ANOVA. Significant differences between treatments were determined using Tukey’s multiple range tests (P≤ 0.05).

Results

Incidence of breast cancer risks were increased dramatically in Bihar (Figure 1). A cumulative increase of 642% was observed in breast cancer cases over a period of ten years.

Figure 1: Number of breast cancer patients visited in the study hospital from different districts of Bihar.

DDT and its metabolites (op-DDT, pp-DDT, pp’-DDE and pp’-DDD) were estimated in blood and tissue samples of breast cancer cases from the different five districts of Bihar viz., Khagaria, Muzaffarpur, Samastipur, Sitamarhi and Vaishal. It was noticed that remarkable variations was observed between the serum and tissue samples. Table 1 shows the mean residual levels of DDT and its metabolites in serum and tissue of breast cancer cases. Total DDT level in serum samples was ranged from nearly 5 ppb to 25 ppb and in tissue samples was ranged from nearly 900 ppb to 4300 ppb.

Figure 2: Mean residual level of DDT in the tissue of breast cancer patients collected from five districts of Bihar.

Maximum accumulated DDT residue in tissue was recorded in Samastipur (total DDT level is 4352 ppb) followed by the Khagaria, Sitamarhi, Vaishal and Muzaffarpur (Figure 2). Figure 3 shows the total DDT residual levels in serum samples. There no remarkable variation was observed in the serum samples between the different districts.

Figure 3. Mean residual level of DDT in the serum of breast cancer patients collected from five districts of Bihar.

Among the DDT metabolites pp’-DDE was accumulated more in both the serum and tissue samples followed by the pp-DDT, op-DDT and pp’-DDD. In serum 9 ppb and in tissue 1109 ppb mean level of pp’-DDE was recorded. pp’-DDE was on average 84.9% and 49.8% of total DDT in serum and tissue respectively (Figure 4). In tissue samples residual level of op-DDT and pp’-DDD was very low.

Figure 4. Residual level of DDT metabolites in serum and tissue of breast cancer patients.

Changes in endogenous sex hormones receptors were observed in breast cancer tissues (Table 2). Significant correlation was observed between the total DDT residual levels in tissues and estrogen receptor. With increasing concentration
The present study indicates that DDT occurrence was positively correlated with the estrogen receptors, but does not show any changes on progesterone receptors in tissues. Hence, DDT caused high impacts on estrogen receptor with a concentration dependent manner. Similarly, Raaschou-Nielsen et al., (2005) observed that higher level of pp'-DDE was associated with the estrogen receptor negative in breast cancers. DDT binds to the estrogen receptor alpha and affects the menstrual cycle in human (Perry, 2006). Estrogen level was highly reduced in the serum of both DDT and DDE accumulated breast cancer patients (unpublished data). Similarly, WHO (2009) signify that both DDT and DDE are reduced the estrogen levels in females. Steinmetz et al., (1996) described the molecular mechanism of action of DDT; DDT binds and activates the estrogen receptor, this complex then interacts with specific DNA sequences in target genes and functions as a transcription factor to enhance (or inhibit) gene expression. Previous reports suggest that EDCs including DDT may affect not only the exposed individual but also future generations. These imply that decipher DDT-specific genes also possibly used as markers for genetic analysis in future. Furthermore, this will help in early detection of cancer.

The present study shows a relationship between the DDT residues and breast cancer risk among Bihar people (India).

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References


**Table 1: Residual range of DDT and its metabolites in blood and tissue of breast cancer patients.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DDT and its metabolites (ppb)</th>
<th>Op-DDT</th>
<th>pp-DDT</th>
<th>pp'-DDE</th>
<th>pp'DDD</th>
<th>Total DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5.0 ± 0.0^a^</td>
<td>5.0 ± 0.0^a^</td>
<td>9.0 ± 8.3^b^</td>
<td>6.2 ± 1.6^a^</td>
<td>10.6 ± 8.4^b^</td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>92.2 ± 120.7^b^</td>
<td>1027.4 ± 746.1^c^</td>
<td>1109.5 ± 795.0^c^</td>
<td>6.6 ± 3.6^a^</td>
<td>2228.8 ± 1495.1^d^</td>
<td></td>
</tr>
</tbody>
</table>

Within rows, mean ± SD followed by the same letter do not differ significantly using Tukey’s test, P≤0.05.

**Table 2: Comparison of total DDT residues with endogenous sex hormones receptors.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Total DDT (ppb) level in individuals</th>
<th>Estrogen receptor</th>
<th>Progesterone receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5.0</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Tissue</td>
<td>8.2</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3.</td>
<td>14.7</td>
<td>Weakly positive</td>
<td>Positive</td>
</tr>
<tr>
<td>4.</td>
<td>24.3</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>5.</td>
<td>5.1</td>
<td>Positive</td>
<td>Positive</td>
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