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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.

Introduction

Breast cancer is one of the most prevalent cancer types among women worldwide. Globally, the incidence of cancer cases is rising every year. There are various types of cancer although breast cancer case has increased 47% during the period from 1975 to 1990 worldwide (Notani, 2001). The number of new cancer cases in the every year was estimated to be around 10 million globally and more than half of these cases were reported to originate from developing countries. In India breast cancer is the second most common cancer after cervix cancer in women, accounting for 19% of the total cancer case (Notani, 2001). Nearly 75,000 new cases of breast and cervical cancers cases are reported in Indian women every year (Bobba and Khan, 2003). Generally, age, sex, and socio-economic status are well known risk factors for occurrence of cancer. Earlier epidemiological studies have suggested that early menarche and late menopause, low parity, obesity, high fat diet, excessive alcohol intake, exposure to ionizing radiation and inherited mutation (via BRCA-1 and BRCA-2 genes) factors are associated with the breast cancer risks (Notani, 2001). Due to environmental carcinogens, especially endocrine disturbing chemicals (EDCs), breast cancer incidences increased during the last 50 years in the industrialized world (Diamanti-Kandarakis et al., 2009). It has been hypothesized that the significance of EDCs carcinogenesis is increased but the molecular mechanisms are still unknown in humans.

Dichlorodiphenyltrichloroethane (DDT) is an insecticide belonging to the class of organochlorine pesticide. DDT is a man-made chemical and does not naturally occur in the nature. Following chemicals are metabolites of DDT, found in environment as well as in some living systems; o,p-DDT (dichlorodiphenyltrichoroethane),p,p'-DDE (dichlorodiphenyldichloroethlene) and p,p'DDD (dichlorodiphenyldichloroethane). Since the discovery of DDT pesticide, usage is dramatically increased worldwide in agriculture and public health. Due to the ill effect and nature persistence of DDT in the environment, United States Environmental Protection Agency (EPA) banned the use of DDT (Arora et al., 2009).

It however, still used in restricted quantities for malaria and other vector control programs in developing countries including India. India is known as a lead producer and largest consumer of DDT (UNEP, 2008).

In 2006, 40,000 metric tons of chemical pesticides were used in India (ENVIS-NIOH, 2007). In general, Tan and Mohd (2001) described that most toxic pesticides create some risk to humans, animals, or the environment.

According to Calle et al., (2002) some epidemiological studies have linked organochlorine toxic pesticides as EDCs with several hormone-related cancers including breast cancer. DDT is a well known EDC and also acts as hormonal carcinogens in rats (Diamanti-Kandarakis et al., 2009). Recently, WHO (2009) is reported that DDT and its metabolite DDE are possible human carcinogens.

Bihar is one of the poorest states of India. Breast cancer incidences continue to be an important public health problem in Bihar.

Major sections of population of Bihar is directly or indirectly associated with DDT exposure through the mosquito vector control programme.

Early diagnosis is also a preventive strategy in cancer control programme.

The aim of this study is to determine whether the breast cancer cases are correlated with uses of DDT pesticide in Bihar.

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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.

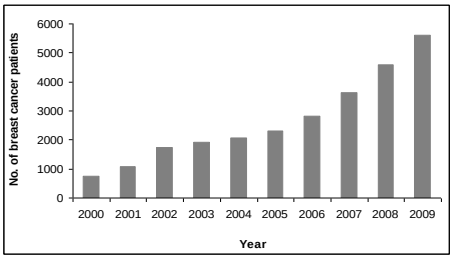
Statistical analysis

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 ($P \leq 0.05$).

Results

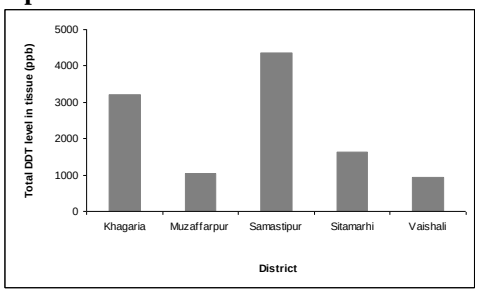
Incidence of breast cancer risks were increased dramatically in Bihar (Figure 1). A cumulative increase of 642% was observed in breast cancer cases over a period of ten years.

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



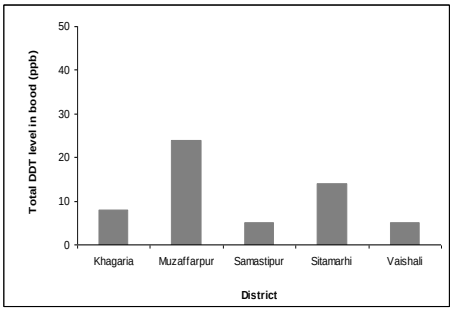
DDT and its metabolites (op-DDT, pp-DDT, pp'-DDE and pp'DDD) were estimated in blood and tissue samples of breast cancer cases from the different five districts of Bihar viz., Khagaria, Muzaffarpur, Samastipur, Sitamarhi and Vaishal. It was noticed that remarkable variations was observed between the 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.



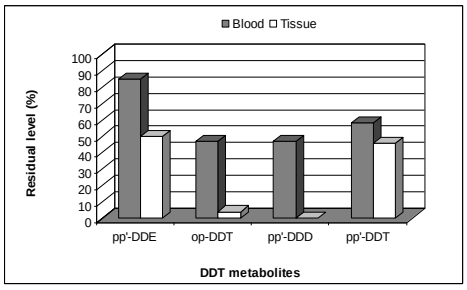
Maximum accumulated DDT residue in tissue was recorded in Samastipur (total DDT level is 4352 ppb) followed by the Khagaria, Sitamarhi, Vaishal and Muzaffarpur (Figure 2). Figure 3 shows the total DDT residual levels in serum samples. There no remarkable variation was observed in the serum samples between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

of DDT residues in tissues was directly proportional to the estrogen receptors disappearance in tissues. There is no remarkable linking between the progesterone receptors and total DDT residues in both serum and tissue samples.

Discussion

Early diagnosis of cancer is a primary strategy in cancer care programme. Many advances have taken place in the treatment of cancer worldwide. Cancer risk factors are arising every year. In India cancer incidence patterns vary according to Notani (2001). In the present study it was observed that breast cancer incidence increased every year in Bihar state (India). This indicates that EDCs including pesticides, industrial facilities and cosmetics from environmental sources may cause more breast cancer risks as hormonal carcinogens in the developing India according to the Diamanti-Kandaraks et al., (2009). Much attention has been made in pesticide residue analysis in human biological samples to assess their risks in human health. Occurrence of DDT residues in the breast cancer peoples of Bihar was observed in the present study. High residual level of DDT and its metabolites are found in the tissues and non significant level of residues was found in the serum. In the last two decades, Arora et al., (2009), Mathur et al., (2005), Chand et al., (1991), Bhatnagar et al., (1992), Srivastava et al., (1995) and Subramaniam and Solomon (2006) have reported that DDT and its metabolites residues are found in samples of human blood in India.

Generally, pesticides can enter into human body by accidental ingestion, inhalation or absorbed through skin contact. According to Ceron et al., (1995) organochlorine insecticides are potentially toxic, highly persistent and easily accumulate in tissues of human body. Hayes et al., (1971) reported that high concentrations of DDT usually get accumulated in adipose tissue than in other tissues. Nearly, 20 ppb and 4000 ppb level of DDT residue was accumulated in blood and tissues respectively of diagnosed breast cancer patients. This implies that DDT was initially found in blood and migrated into tissues. Among DDT metabolites, pp'-DDE concentration was higher in the tissues. Because pp'-DDE has high fat: water partition coefficients therefore, it tends to accumulate in adipose tissue (Klaassen, 1990). According to WHO (2009), detection of lower ratio of DDD or DDT to DDE indicate long-term exposure. Cocco et al., (2000) reported that higher accumulation of DDE levels in adipose tissue had increased breast and liver cancer risks. Recently, WHO (2009) described the metabolic pathway of DDT in rodents and humans. On that metabolic pathway DDT is initially metabolized in the liver to intermediary pp'-DDE and DDA metabolites. Usually, in biological systems DDT is rapidly converted in to DDE and DDE is slowly converted into DDA. Many scientific investigators have reported that DDT act as carcinogen in rodents. DDT was accelerating the mammary gland tumors in rodents as xenosterogens (Scribner and Mottet, 1981). Recently, WHO (2009) stated that DDT and DDE are carcinogenic to animals. The present study implies accruing evidence that accumulation of DDT and its metabolite residues in mammary tissue may contribute to carcinogensis in breast.

Generally, EDCs modulates sex hormones as agonists or antagonists or as mixed effects and can cause reproductive cancer risks. DDT was known as a xenoestrogen in humans (Fleseriu, 2010). An endocrine disruptor can injure or destroy an organ that has the task of supplying hormones (Schettler, 1999). 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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Table 1: Residual range of DDT and its metabolites in blood and tissue of breast cancer patients.

Sample	DDT and its metabolites (ppb)				
	Op-DDT	pp-DDT	pp'-DDE	pp'DDD	Total DDT
Blood	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	9.0 ± 8.3^{b}	6.2 ± 1.6^{a}	10.6 ± 8.4^{b}
Tissue	92.2 ± 120.7^{b}	1027.4 ± 746.1^{c}	1109.5 ± 795.0^{c}	6.6 ± 3.6^{a}	2228.8 ± 1495.1^{d}

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.

S.No.	Total DDT (ppb) level in individuals		Estrogen receptor	Progesterone receptor
	Blood	Tissue		
1.	5.0	4652.4	Negative	Positive
2.	8.2	3302.8	Negative	Positive
3.	14.7	1928.4	Weakly positive	Positive
4.	24.3	1135.6	Positive	Positive
5.	5.1	975.2	Positive	Positive