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Research Paper

Biomonitoring of DDT in Blood and its Toxicity may lead to Breast Cancer

Biomedical

KEYWORDS : DDT, Breast Cancer, Toxicity

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ABSTRACT

The extensive use of DDT globally in 1940s and successive decades has contaminated the environment which then comes in human blood and tissue through food chain. It has ability to damage human genetic material which further leads to disease like a cancer. The main objective of the present study was to bio-monitor DDT and its metabolites in blood samples of breast cancer patients and to evaluate this pesticide as a possible risk factor. DDT and its metabolites were measured in the blood samples of breast cancer patients as well as in healthy women. Estimation of DDT and its metabolites were done by Reverse Phase High Performance Liquid Chromatography (RP- HPLC).

In Summary, results obtained from the present study indicate DDT as a possible biomarker for the DDT exposure in Bihar, which may lead to breast cancer among women who are professionally or environmentally exposed to this insecticide in this region.a

Introduction-

Increased breast cancer incidence has been reported from the various part of the world. It comprises 18% of all female cancers (McPherson, 2000). The risk to develop breast cancer is influenced by several environmental factors and it has also shown to associate with pesticides (Woff et al. 1993) although some researchers reported genetic factor to a lesser extent for the development of breast cancer. Pesticides especially organochlorine play a significant role in the proliferation of breast cancer. Organochlorines have been shown to associate with neurological disorder (Gladen et al. 1996) such as Parkinson's disease (Chhillar et al. 2013, Fleming et al. 1994), Attention deficit hyperactivity disorder (Sagiv et al. 2010) etc. The route of exposure of DDT is mainly through food chain. Once it comes into food chain, it biomagnifies and concentrates more at the apex of food chain. The increased incidence of breast cancer in Bihar needs to an extensive study in this region.

Human health or disease depend more upon environmental factors than the genetic factor. Prevalence of pesticide use in India as well as in world has unintended effect on the human being. Immense use and exposure of pesticides through different sources has led to potential health effects such as cancer. Incidence of cancer is being significantly increasing every year all over the world. It is now evident that pesticides can disrupt endocrine receptors, alter hormone metabolism, act as tumor promoter, and ultimately lead to diseases like cancer. Further severe effect of pesticides may damage chromosome, DNA and histone proteins or indirectly impair cellular organelles like endoplasmic reticulum and mitochondria, endocrine system, nuclear receptor etc.(George and Shukla, 2011; Rakitsky et al. 2000, Mostafalou and Abdollahi, 2013).

Although, different types of cancer are being cured by various drugs, the root cause is still very meagre and research is going on in this area to find out more details.

DDT (1, 1, 1 –trichloro 2, 2 –bis (2 chlorophenyl-4-hlorophenyl) ethane) was banned in U.S. in 1972 besides the use of public health purpose and was totally banned in the year 1979 (Kang and Chang, 2011). Though it is banned in several countries but due to its bioaccumulation and biomagnification properties, it is still found in food especially of animal origin. Interestingly, production of DDT is still continued in India, China and Democratic People's Republic of Korea for the elimination or control of diseases vector in these three countries. The total production of DDT in India in the year 2007 was 6300 t (UNEP, 2008). These DDT residues are highly persistent and come in human body through food chain. They have tendency to accumulate in lipid rich tissue inside the body.

The aim and objective of the present study was to assess the DDT and its metabolites in blood samples of breast cancer patients as a possible risk factor for the same disease in Bihar.

Materials and Methods-

150 breast cancer patients and 115 normal women were selected randomly for the present study. Background informations of breast cancer patients were obtained and categorized into age, district, breast cancer types, stage and menopause status wise. In order to estimate the *p, p'* –DDT, *p, p'* -DDD and *p, p'* –DDE, blood samples were collected from the breast cancer patients and from normal women with their consent at Mahavir Cancer Institute and Research Centre, PMCH, NMCH, Patna. Blood samples were centrifuged at 3000 rpm for 15 minutes and serum samples were collected and kept at -80^{0}C until further use. The present study has been duly approved by Institutional Human Ethical Committee of Mahavir Cancer Institute and Research Centre, Patna.

Estimation of pesticides was done by using RP- HPLC (Reverse-Phase High Performance Liquid Chromatography). The column was C18 silica gel pack. Methanol and water was used as a mobile phase and mode of operation was isocratic elusion. The extraction of pesticide was performed by the protocol followed by Subramanian and Solomon (2006).

Statistical analysis-

Mean and standard deviation of selected organochlorine pesticides in blood samples of breast cancer patients were calculated using basic statistics. Further, Statistical calculations were performed by using unpaired Student *t* test between normal and selected organochlorine level in blood and tissue samples of breast and ovarian cancer patients. Relative risk assessment were obtained in order to determine the correlation between levels of selected organochlorine pesticides for women health in the same samples and its 95% confidence intervals(CI) were also calculated.

Results-

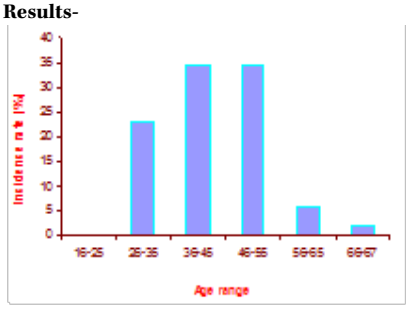

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Figure1: Breast cancer incidence among different age groups

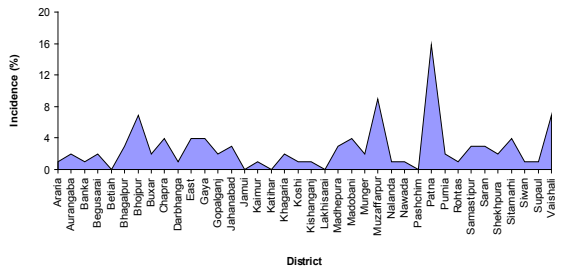


Figure2: Prevalence of breast cancer in different districts of Bihar.

The figure1 illustrates the breast cancer incidence among different age groups of women. Maximum incidence was recorded nearly 35% among 36-55 years of age group of breast cancer patients and it was not found surprisingly among the 16-25 years of age group of women.

The figure2 illustrates the breast cancer incidence in different districts of Bihar. Breast cancer incidence was found remarkably high in Patna, Muzaffarpur, Bhojpur, and Vaishali districts of Bihar. Women with stage III of breast cancer were observed more followed by stage II, Stage IV and Stage I (figure3). The percentage if incidence for breast cancer Stage I, II, III, and IV has found 12, 19, 55 and 14 respectively.

Among different types of breast cancer, Ductal Carcinoma in Situ was found more (50.5%) among breast cancer patients than the other cancer types. Infiltrating Duct Carcinoma was found as the second most prevalent type of breast cancer (33%) (Table: 1).

Mean and standard deviation of the selected organochlorine pesticides in blood of breast cancer patients has shown in table: 2. Mean level of *p, p'*- DDT, *p, p'* – DDD and *p, p'* –DDE were found 17.71, 22.95, and 25.38 respectively in blood samples of breast cancer patients, which were nearly two to three folds higher than the normal level i.e. 9.59, 11.77 and 8.63 respectively.

In the present study frequency of premenopausal breast cancer women has been recorded slightly high than the postmenopausal women (Figure: 4).

Table1: Percentage of breast cancer types

Cancer Types	Percentage
Ductal carcinoma in situ	50.5
Infiltrating duct carcinoma	33
Others carcinoma	16.5

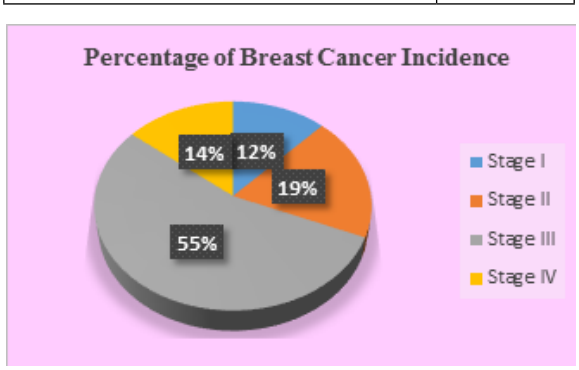


Figure3: Percentage of breast cancer incidence among different stages.

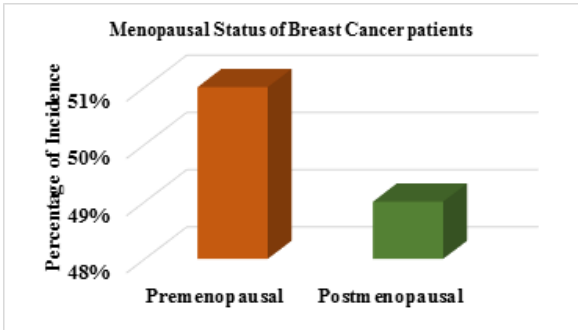
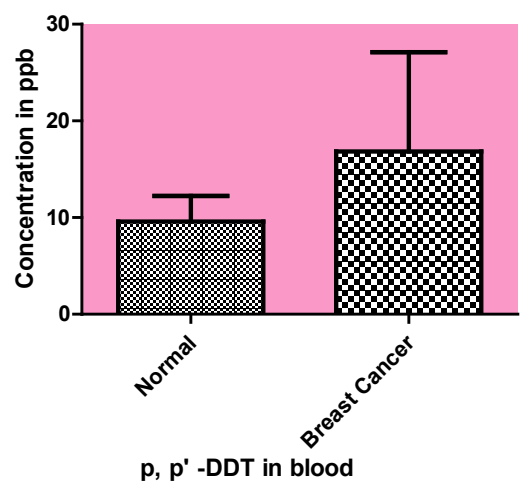
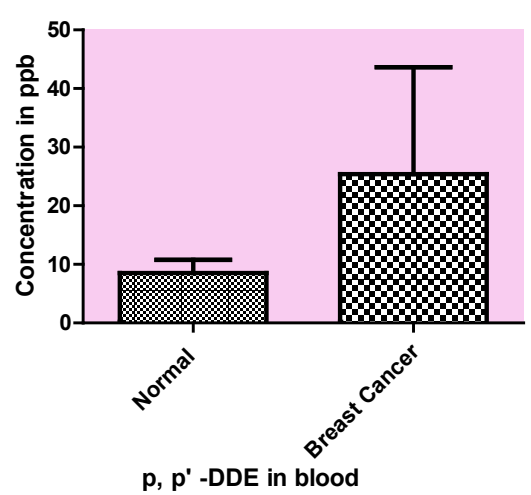


Figure4: Frequency of menopausal status of Breast cancer patients.

Table2: Level of DDT and its metabolites in blood of breast cancer patients.

S. No.	Pesticides	Mean ± SD (ppb)		p- value
		Normal	Blood	
1.	***p, p'* –DDT**	9.59± 2.65	17.71± 10.16	≤0.05
2.	***p, p'* –DDD**	10.77 ± 4.69	21.95 ±16.03	≤0.05
3.	***p, p'* –DDE**	8.63 ± 2.19	25.38 ± 18.27	≤0.005





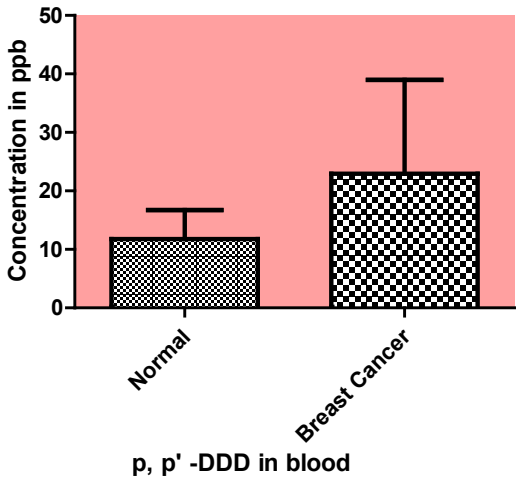


Fig5: Level of *p, p'*DDT, *p, p'*- DDE and *p, p'* -DDD in blood samples of normal women and breast cancer patients.

Bar diagrams represent the mean values and its significance in blood samples of breast cancer patients (Figure: 3). Two tailed unpaired t-test p –value for *p, p'*- DDT, *p, p'* – DDD and *p, p'* – DDE was found ≤0.05, ≤0.001 and ≤0.005respectively.

Table 2: Values of relative risk for breast cancer.

Metabolites of DDT in breast cancer patients	Relative Risk	95%CI
p, p'-DDT	0.90	0.59-1.3
p, p'-DDD	1.31	0.98-1.77
p, p' -DDE	1.41	0.98-2.02

Table 2 shows the relative risk values calculated for normal and breast cancer patients in blood samples. Among all three metabolites of DDT studied for breast cancer, highest relative risk value was obtained as 1.31 for p, p' -DDD in blood samples.

Discussion-

Several risk factors have been reported for breast cancer such as age, alcohol intake, family history etc. Few researchers support that breast cancer increases with age (Moser et al. 2007) and then decreases after the age of 50 years (Petronella, 1993). In present study it has been observed that breast cancer is most common in age group of 36-55 years and then decreases gradually, indicating that prevalence of breast cancer cases occurs mostly in the reproductive age group. The exact cause is still not known but it may be due to prolonged exposure to pesticides. In US the risk of breast cancer proportionally increases with age of female. Risk to develop breast cancer at the age of 70 years is more than the lower age groups of women (Facts & figures, 2011-2012).

Indiscriminate use of DDT in Bihar has continued since 1976 for the control of vector of malaria and visceral leishmaniasis or Kala-azar (Kaul et al. 1994) mainly. In the year 2003 and 2004 in Bihar DDT spray inside the houses and cowshed have been reported (Thakur, 2007). Recently in India, the annual allocation of DDT in 2006-07 for Indoor Residual Spraying (IRS) was 6450 metric tonnes (MT) and 375 MT for elimination of malaria and Kala-azar respectively (NVBDCP, 2007). More recently, in the year 2014 IRS is being continued and the districts where DDT resistance has been previously reported, other insecticides are being used (NVBDCP, 2014).

DDT level in water (Meybeck et al. 1989), soil (Dua et al. 1996; Mishra et al. 2011), air (Ramesh et al. 1989), animal feed (Mukherjee and Gopal et al. 1995), mother's milk (Kumar at al 2006) has been reported previously in India. In the year 2013, DDT and HCH residues was reported in animals and mother's milk in Uttar Pradesh, India (Maurya et al. 2013). Although DDT and its metabolites in blood samples (Agarwal et at. 1976) have been reported previously in India, the data of body burden of this pesticide especially in breast cancer patients of Bihar are very meagre.

Prevalence of breast cancer in the present study was recorded mostly in the endemic districts of Bihar which include Patna, Vaishali, Muzaffarpur and Bhojpur mainly.

It has been hypothesized that DDT and its metabolites binds to estrogen receptors and it works via activation of transcription and combined with transcription factor AP-1 and activated AP-1-mediated gene transactivation through both ER-dependent and ER-independent pathways. DDT and DDD have shown most potent activators of AP-1 activity (Frigo et al. 2002). Some researcher has reported that DDT mimics epithelial growth factor (EGF) and induced proliferative change in Estrogen Receptor (ER) negative breast cancer cells through a non- estrogenic pathway (Shen and Novak 1997a).

Weak associations between blood DDT and DDE levels and miscarriages (Gerhardt et al. 1998) and infertility (Gerhardt et al. 1999) has been reported previously. Some studies have been shown the association of levels of organochlorine pesticides and breast cancer (Kleanthy and Andreas, 2009, Calle et al. 2002, Boada et al. 2012).

Relative risk assessment has been studied in the present study and a strong correlation between level of *p, p'* –DDE in blood samples of breast cancer patients has been observed which indicates an increased risk from the same pesticide on the female reproductive health.

Summary:

Present study supports that age is a significant risk factor for the development of breast cancer. DDT and its metabolites has been detected in the blood samples of breast cancer patients although they are banned in few years ago but still being used restrictedly for the disease vector control. The elevated level of DDT and its metabolites in the breast cancer patients indicate that it may be one of the factors for increased incidence of breast cancer in this region. Therefore, increase of awareness about the age of women and environmental contaminants like DDT is necessary in Bihar to combat breast cancer.

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