Studies on Identification of Organochlorine pesticide residues and their concentrations in Breast cancer patients serum sample by GC-MS

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Abstract: Many social and lifestyle changes have occurred in the decades following World War II, a period during which the breast cancer rates increased dramatically. In addition, and strikingly, the increasing incidence of breast cancer over these decades paralleled the proliferation of synthetic chemicals. Many of these chemicals with lipophilic nature persist in the environment accumulate in the body. These lipophilic pesticides may increase cancer risk through their persistent accumulation in adipose tissue, slow release into the blood and peripheral tissue, and eventual impact on the endocrine system. In the present study qualitative and quantitative organochlorine pesticide residues where analyzed and increased risk of breast cancer was observed.

I. INTRODUCTION

The effects of pesticides on human health are more harmful based on the chemical persistence and the length and magnitude of exposure (Lorenz, 2009). Owing to the environmental impacts of the pesticides and following the general parameters of toxicity persistence and impact, several priority lists called “red” and / or “black” list have been published (Gabaldon *et al*, 1999; Deepthi, 2010). Among the various classes of pesticides that have been used in agriculture, the organochlorine pesticide group is major and is lipophilic in nature, potentially toxic, highly persistent and resistant to biodegradation and readily accumulates in the human body, causing a variety of health hazards (Subramaniam *et al*, 2006; Govindaraj *et al*, 2010; Boada *et al*, 2012). The presence of organochlorine and organophosphorous pesticides has been reported in edible crops, fruits, soil, milk and other consumer products (Sukesh *et al*, 2012). Due to their high persistence and lipophilicity, these compounds tend to accumulate in lipid rich tissues of the organisms (Tan *et al*, 2008; Waliszewski *et al*, 2005; 2010 and 2011) and get biomagnified through the food chain. The presence of pesticides in the blood and other tissues and the fact that they have longer half lives is an alarming indication of in-vitro exposure of generations to come (Ritter, 1995).

In India the incidence/mortality ratio is 0.48 compared with 0.25 in North America (Parkin *et al*, 2005). Over the years, the incidences of breast cancer in India have steadily increased and as many as 100,000 new patients are being detected every year (Yip *et al*, 2006; Michael *et al*, 2003). A 12% increase was registered by cancer registries from 1985 to 2001, which represented a 57% rise of cancer burden in India (Yip *et al*, 2006; Hadjiiski *et al*, 2006). During the year 2001, nearly 0.80 million new cases were estimated in India and according to Murthy *et al*, (2008), this would increase to 1.22 million by 2016 resulting in a 1.5 increase for all sites of cancer both at the national level as well as the state level as a result of change in size and composition of the population.

Table: 1.1

Comparison of cancer incidence in USA, India and Japan

Cancer	USA		India		Japan	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
Oral cavity	50	11	102	60	29	12
Nasopharynx	4	2	4	3	3	2
Other Pharynx	19	9	57	42	10	7
Oesophagus	31	31	63	59	58	43
Stomach	56	34	43	39	489	225
Colon/Rectum	356	139	40	26	342	143
Liver	30	31	17	16	186	146
Pancreas	72	68	11	11	76	71
Larynx	33	11	35	22	17	5
Lung	463	402	55	51	262	214
Melanoma of skin	113	21	3	1	3	2
Breast	914	212	191	99	314	77
Cervix uteri	78	33	307	174	111	30
Corpus uteri	155	20	17	5	45	13
Ovary etc.	106	62	49	29	66	37
Prostate	1043	179	46	28	111	55
Testis	40	2	6	3	13	2
Bladder	144	28	20	16	56	17
Kidney etc.	86	31	8	6	42	19
Brain, nervous system	54	37	21	16	24	9
Thyroid	46	3	14	4	31	5
Non-Hodgkin lymphoma	135	59	24	19	58	30
Hodgkin's disease	22	4	8	4	3	1
Multiple myeloma	35	26	8	6	16	12
Leukemia	80	54	26	20	48	34
All sites but skin	3223	1391	1017	688	2230	1213

Source: India Cancer Statistics (2011)

The changing lifestyle in the Study area is making women prone to breast cancer. Breast cancer is the most common type of cancer in many cities and the second most common in rural areas (Sambasivaiah *et al*, 2004; Imran Ali *et al*, 2011). The rates of young women being diagnosed for suspected breast cancer is increasing in Andhra Pradesh. Andhra Pradesh was the fifth highest state with deaths due to breast cancer, with 3863 deaths in the year 2010 (Shukla, 2011, He T-T, Zuo *et al*, 2017). Guntur district in the state is increasingly under focus for the increase in the incidence of breast cancer (Table: 1.2).

Table: 1.2

Cancer incidences reported at the Guntur Government General Hospital and Bommidala Cancer Institute, Pedakakani, Guntur, Andhra Pradesh from 2004-12

Disease	2004	2005	2006	2007	2008	2009	2010	2011	2012(May)
Cervix. cancer	292	330	537	312	337	373	363	260	208
Breast. cancer	170	176	179	170	168	157	176	160	170
Oral cavity cancer	62	60	52	56	78	105	75	76	72
Hypopharynx cancer	84	78	83	52	81	81	102	68	53

Oesophagus cancer	14	13	17	13	12	21	33	16	25
Brain cancer	30	35	19	24	25	35	35	26	22
Retinoblastoma cancer	0	0	2	4	0	0	0	0	3
Lung cancer	18	19	17	13	15	17	16	12	17
Bladder cancer	7	15	21	11	10	12	10	05	06
Prostate cancer	3	5	2	12	14	06	09	13	2
Sarcoma cancer	2	0	8	4	2	5	1	4	2
Rectum and anal canal cancer	5	8	6	11	8	15	18	12	14
Spine cancer	11	20	18	6	7	8	7	5	10
Other cancers	13	24	29	19	10	21	31	25	33

Source: Guntur Government General Hospital, Guntur and Bommidala Cancer Institute, Peddakakani, Guntur (A.P.).

Thus in the present study an attempt is made to identify the organochlorine pesticides that may trigger breast cancer.

II MATERIALS AND METHODS

2.1 Sampling and Data Collection:

In the present study, 300 cases of various female breast cancer complaints were selected. Of these 300 cases included in the study, 96 were selected for the year 2008, 87 from 2009 and 117 from the years 2010 and 2011 from the Guntur Government General Hospital and the Bommidala Cancer Institute, Pedakakani, Guntur. Approval was obtained for the recruitment of patients with various breast cancer complaints, the collection of information through a questionnaire, the collection and analysis of breast patient's serum samples was carried out.

Blood samples were collected in residue free heparinized 10 ml glass vials containing 200 USP units of heparin in 0.2 ml solution with the help of sterilized syringe. The labels on the specimen containers contained information regarding the patient's hospital, respondent number (RD) and surname, which was used to obtain pathology details, but upon collection the samples were assigned a breast cancer- respondent code (BC-RD) number.

2.2 Extraction from Blood:

Extraction was based on the method followed by Agarwal *et al*, (1976), with slight modifications. Blood (5 ml) was diluted by adding 25 ml of distilled water and 2 ml of saturated brine solution was added and transferred to a 125 ml capacity separatory funnel and extracted with hexane: acetone (1:1) (20ml) (thrice) by shaking the separatory funnel vigorously for 15-20 minutes by releasing the pressure intermittently. The layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulphate and concentrated to about 1-2 ml using a rotary vacuum evaporator.

2.3 Clean up procedures:

The process of sorption was carried out in chromatographic columns of alumina (Holden et al, 1969 and silica (Kadoum, 1967; 1968). Florisil, Alumina and Silica Columns.

The alumina column was used for the separation of tissue debris from pesticides which was used by Holden and Marsden (1969). The column was made of glass having length of 40-42 cm with an internal diameter of 6 mm. The column was filled with 2 grams of the florisil of 0.3 micron size (already activated at 180^{o}C for overnight in a hot air oven and then partly deactivated by shaking with 5% by weight of water). The concentrated extract was re-dissolved in l ml of n-hexane (fractionated) and transferred to the surface of the florisil column. Thereafter, the pesticides adsorbed on the column were eluted with 12 ml of n-hexane and the eluted samples were then reduced to 1 ml by the rotary vacuum evaporator and were passed through a new column. The new column of the same size was packed with 2 grams of silica gel for column chromatography No. 60, 0.060 millimetre size (activated at 120^{o}C for 2 hours, cooled and deactivated with 3.5% distilled water). For the removal of traces of moisture a layer of activated Na_2SO_4 was set on top of the silica gel. The elution of pesticides was done by n-hexane and Acetone 3:1, 1:1, 1:3.

2.4 Analysis of organochlorine pesticides using Gas Chromatography-Mass Spectroscopy:

The organochlorine pesticides α, β, γ, δ HCH, alachlor, aldrin, α, β - endosulfan, endosulfan SO_4, dieldrin, DDT, DDD, DDE and organophosphate pesticide monocrotophos, atrazine, phorate sulfone, chlorpyrifos, ethion, malathion and phosphamidon in the breast adipose tissue was extracted and analysed by gas chromatography. Peak identification was performed by the GC – MS software. Calibration table was set up with a relative retention time (RRT). The following conditions were followed for the analysis (Table: 2.1).

III RESULTS

3.1 Organochlorine Pesticides in Blood Samples:

Among the thirteen organochlorine pesticides and their isomers analysed in the tissue samples, the mean levels of isomers of Hexa Chlorocyclo Hexane (HCH) for benign group and

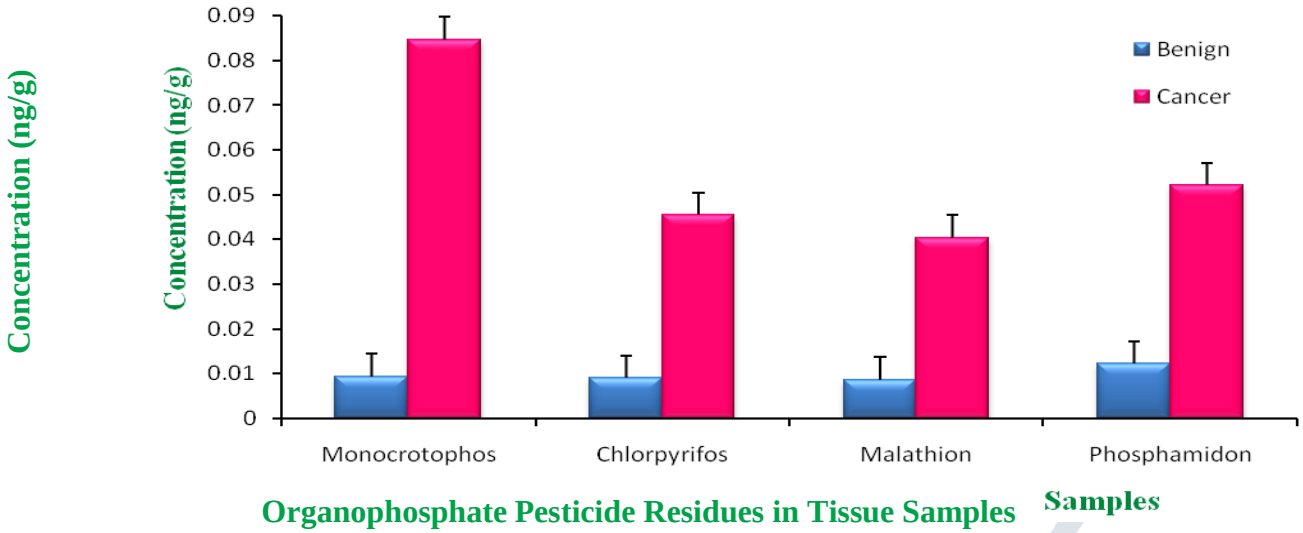
Table: 2.1

Conditions for analysis of organochlorine and organophosphate pesticides using Gas Chromatography-Mass Spectroscopy

Conditions	Organochlorines	Organophosphates
Detectors	Electron capture	Nitrogen Phosphorus
Oven temperature (^{o}C)	290	270
Detector temperature (^{o}C)	290	280
Injection temperature (^{o}C)	280	280
Flow rate (ml/min) a. Carrier gas b. Make-up gas	 1.0 40	 2.0 30
Total run time (Minutes)	29.64	29.64

Figure: 3.1

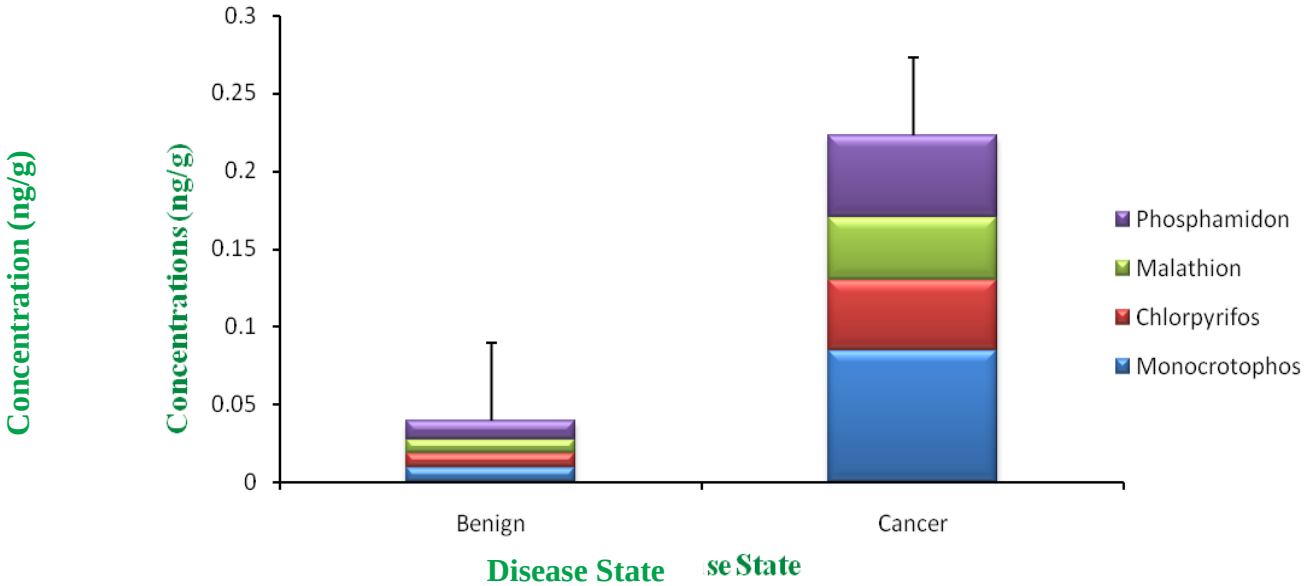
Comparison of organophosphate pesticide residue concentrations in the tissue samples among benign and cancer groups



the true cancer group were (α- HCH – 0.0096 and 0.0484, β- HCH – 0.0162 and 0.0196, γ- HCH – 0.0136 and 0.0224, δ- HCH – 0.0006 and 0.0038) and were statistically significant except β- HCH (p= 0.0001, 0.072, 0.000 and 0.0000) (Table: 3.1). Endosulfan and its isomers were quantified in both benign group and true cancer group. The mean levels of isomers of Endosulfan in benign group and true cancer were (α- endosulfan- 0.0044 and 0.0159; β- endosulfan – 0.0000 and 0.0099; Endosulfan sulfate – 0.0000 and 0.0019) and were statistically highly significant (p = 0.000; 0.000; 0.000).

Figure: 3.2

Comparison of specific concentrations of individual organophosphate pesticide residues in the tissue samples that make up the total with respect to benign and cancer groups



Dichlorodiphenyltrichloroethane (DDT) was identified in benign group and true cancer patient's blood samples at mean levels of (0.015 and 0.0150) and with p value of 0.0000 which is also highly significant. A major metabolite of DDT, i.e., 2, 2-bis (*p*-chlorophenyl) -1,1-dichloroethylene (pp'DDE) was traced at mean levels of 0.0201 and 0.0189 (p = 0.000) in the samples analysed. DDE is more persistent than DDT. 2, 2-bis (p-chlorophenyl) -1,1-dichloroethane (DDD), another metabolite of DDT, was traced at mean levels of 0.0052 and 0.0201 (p = 0.000).

Aldrin was quantified in the samples analysed at mean levels of 0.0025 and 0.0066 (p = 0.000) and Dieldrin (banned with effect from May, (1990) was traced in the samples analysed at mean levels of 0.0029 and 0.0085 (p = 0.000). Alachlor was also traced in both benign and cancer groups with mean values of 0.0022 and 0.0064 (p = 0.0000), which was statistically highly significant. All the data comparatively have shown a significant difference between the two groups except β-HCH which was not significant. The organochlorine pesticide residue concentrations in whole blood samples were compared between true benign and true cancer. The mean concentration of pesticides was high in the blood samples of women diagnosed with breast cancer compared to the benign group. There was a statistical difference between the mean concentrations of pesticides between benign and cancer groups.

Table: 3.1
Comparison of mean values of organochlorine pesticide residues in the blood samples among the benign and cancer groups by Paired t-test

Parameter	Disease Group	Mean ± S.D	*p*-value
α- HCH	BENIGN	0.0096 ± 0.0132	0.0000000
	CANCER	0.0484 ± 0.0291	
β- HCH	BENIGN	0.0162 ± 0.0154	0.0715347
	CANCER	0.0196 ± 0.0110	
δ- HCH	BENIGN	0.0006 ± 0.0024	0.0000033
	CANCER	0.0038 ± 0.0055	
γ- HCH	BENIGN	0.0136 ± 0.0061	0.0000000
	CANCER	0.0224 ± 0.0106	
Alachlor	BENIGN	0.0022 ± 0.0036	0.0000000
	CANCER	0.0064 ± 0.0038	
Aldrin	BENIGN	0.0025 ± 0.0046	0.0000000
	CANCER	0.0066 ± 0.0049	
α- **Endosulfan**	BENIGN	0.0044 ± 0.0063	0.0000025
	CANCER	0.0159 ± 0.0197	
Dieldrin	BENIGN	0.0029 ± 0.0043	0.0000000
	CANCER	0.0085 ± 0.0063	
o.p.DDD	BENIGN	0.0052 ± 0.0046	0.0000000
	CANCER	0.0201 ± 0.0119	
p.p.DDE	BENIGN	0.0189 ± 0.0181	0.0000000
	CANCER	0.1116 ± 0.1036	

III	β- **Endosulfan**	BENIGN	0.0000 ± 0.0000	0.0000300
		CANCER	0.0099 ± 0.0200	
	p.p.DDT	BENIGN	0.0015 ± 0.0043	0.0000000
		CANCER	0.0150 ± 0.0145	
	Endosulfan.SO4	BENIGN	0.0000 ± 0.0000	0.0000014
		CANCER	0.0019 ± 0.0034	

DISCUSSION

Environmental pollutants have been identified as potential inducers of breast cancer because many of these compounds have estrogen-like traits. Some of the most common and well-studied environmental pollutants are organochlorines. These include: DDE (1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene), DDT (Dichloro Diphenyl Trichloroethane), DDD (Dichloro Diphenyl Dichloroethane), α, β, γ, δ- HCH (Hexa Chlorocyclo Hexane), α, β- endosulfan, endosulfan sulphate, alachlor, aldrin and dieldrin.

Organochlorines (or-GAN-oh-KLOR-eens) are a group of first generation chemicals commonly used as pesticides. Higher levels of estrogen in the blood were linked to an increased risk of breast cancer (Zhang *et al*, 2013, He T-T, Zuo et al, 2017). In the present study it was observed that there was a potential for organochlorine pesticides to be the risk factor for breast cancer by measuring the levels of a series of organochlorine pesticide residues in the tissue and blood samples from the study population with breast cancer development, and identified the presence of endosulfan, DDT, HCH with the highest concentrations (Table: 3.1). The levels of β-HCH were higher in the biopsy tissue taken from women with invasive cancer compared with the benign breast biopsies but this was not statistically significant (p=0.06). The levels of the organochlorine pesticides identified in the study were fairly consistent with the findings depicted in the previous studies suggesting a strong correlation between serum and adipose tissue concentrations of various organochlorines (Jennifer *et al*, 2005; Xu *et al*, 2010; Boada *et al*, 2012), and strong correlations between various adipose tissue (Ejaz *et al*, 2004; Jennifer *et al*, 2005). However, some studies have not observed similar associations (Mozzachio *et al*, 2008, He T-T, Zuo et al, 2017).

IV CONCLUSION

The concentrations of several organochlorine pesticide residues (OCPs) were identified in both blood samples of benign and cancer groups. The mean levels of these pesticides were highly significant when compared to the levels of β-HCH (Table: 3.1). The total content of DDT, DDD and DDE in blood samples of cancer group were high compared to benign group (Figure: 3.1,3.2). Total-endosulfan (α, β and endosulfan sulfate) levels were slightly higher in the cancer group than in benign group and showed high loading factor correlation (Table: 3.1). Alachlor, aldrin and dieldrin were also identified and showed significant difference between benign and cancer groups (Table: 3.1).

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