Original Article

Organophosphate and Pyrethroid Residues in the Milk of Women and Breast Cancer Patients from Karachi

1. Kiran Ajmal 2. Muhammad Ahmed Azmi 3. Ijaz Hussain Zaidi 4. Syed Naeemul Hasan Naqvi 5. Rehana Perveen 6. Muhammad Arshad Azmi

1. Sen. Lecturer BIPS, Karachi 2. Prof. of Physiology Al-Tibri Medical College Karachi 3. Asstt. Prof. of Pharmacology Al-Tibri Medical College Karachi 4. Prof. of Pharmacology BIPS Karachi 5. Prof. of Zoology University of Karachi.

ABSTRACT

Aim: The aim of this study was to determine the presence of pesticide residues organophosphate and pyrethroid in the milk of women and in serum of breast cancer patients from different localities of Karachi due to pesticide exposure.

Study Design: An experimental study.

Place and Duration of Study: This is a research-based study that was conducted in the Department of Pharmacology, Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi from March 2008 to March 2010.

Materials and Methods: A total 40 milk samples were collected from private clinics and 6 serum samples from breast cancer patients were also collected from private cancer hospitals based at different areas of Karachi. All the samples were analyzed for the presence of pesticide residues. Samples of milk and serum were prepared accordingly and the purified samples were injected into the HPLC apparatus. The peaks of the samples were compared by the retention time of the standard peaks. The chromatogram obtained indicated the quantity of pesticide residues.

Results: Milk samples and serum samples were analyzed using HPLC technique. Pesticides such as malathion, permethrin, deltamethrin and Polytrin-C were detected in different concentrations. The levels were significantly higher than the maximum residual limit.

Conclusion: It is concluded that the presence of pesticides in the human body is a major concern in the development of various ailments because of possible immunotoxic, mutagenic and carcinogenic potential of pesticides.

Keywords: Organophosphates, Pyrethroids, Human Milk, Breast Cancer, Pesticide Residues.

INTRODUCTION

Pesticides comprises of various chemicals that are deliberately added in the environment for the purpose of eliminating different pests. The main consumption of pesticides is in the agriculture field. In Pakistan large number of populations used synthetic pesticides to save their products. These synthetic pesticides are causing various problems e.g., pesticidal pollution, resistance in pests and accumulation of pesticide residues in the body of animals and human beings. The use of pesticides at one side is important in eliminating the pests but on the other hand produce risk to the human health when used indiscriminately without any proper apparel. Many researchers have reported that the presence of pesticides in the samples of human body fluids and tissues is a major concern from the health point of view because of immunotoxic, carcinogenic and mutagenic nature of the pesticides.

The purpose of this study was to determine and quantify the different pesticide residues in human milk and serum samples of breast cancer patients from different hospitals and clinics of Karachi. The genotoxic or clastogenic potency of lactational exposures to DDT and HCHs in infants described that the frequencies of SCE control and SCEANF showed increasing and decreasing tendencies with the increasing exposure to DDT and also HCHs through the breast milk[1]. The lactational transfer of presumed carcinogenic and teratogenic organochlorine compounds within the first six months of life explained that these compounds are prenatally transmitted from mother to fetus and mother's milk due to its high lipid content[2]. The simultaneous presence of DDT and pyrethroid residues in human breast milk from three town of South Africa for malaria control program has also been reported[3]. It is also indicated that the environmental contamination with organochlorine pesticides may be related to the risk of breast cancer in rural Victoria, Australia and in this respect no significant correlation was noticed between organochlorine contamination and the age-standardized rate of breast cancer across all the regions of Victoria, Australia[4]. Activation and proliferation of breast cancer tumor by estrogenic microenvironment generated by organochlorine residues in adipose tissues adjacent to breast carcinoma may be affected[5]. The high levels of organochlorines in mother's milk from Chennai city, India indicated that the children are at a higher health risk in this city as these chemicals may transfer from

mother's milk through feeding[6]. Human milk of mother living in Northern Tunisia has been assessed for organophosphate residues and found that the concentration of OCPs increased with mother's age and decreased with the number of children[7]. Keeping the hazardous effects in priority, present work was under taken into consideration from the persons of different areas of Karachi. These females are living in highly polluted areas. As pesticides accumulated via food chain, these persons are not adopting precautionary measures in handling the pesticides or misuse of pesticides without knowing their adverse effects and so their health may be affected. Therefore, it is suggested that persons living in the highly polluted areas should be well informed and educated by the controlling authorities not to use synthetic pesticides but encourage them to use phytopesticides. This is the main aim of the present study.

MATERIALS AND METHODS

Selection of Stations: Milk samples were collected from a private maternity clinic located in Shireen Jinnah colony present in the rural area of Gadap, which is approximately 1.5 km far from Baqai Medical University, Fatima Hospital based at Baqai Medical University and a few samples were collected randomly from different areas of Karachi. Serum samples of breast cancer patients were also collected form a private cancer hospital of Karachi.

Collection and storage of sample: Human milk samples i.e., 2-4 ml and 1 ml serum sample from breast cancer patients were also collected in glass vials from different hospitals. All the samples were kept in freezer for analysis.

Pesticide determination: The milk and serum samples were subjected to fat extraction method[10]. The process of sorption was conducted in chromatographic column of alumina[10] and silica[11]. The processed extract was evaporated by placing on a shelf without cover to obtain 1 ml of sample. Pesticide residues in the samples were determined by using high performance liquid chromatography (HPLC) technique. A packed column (Zorbax NH_2) a polar bound phase with particle size of about 7 micrometer in diameter. A mobile phase n-Hexane was used with a flow rate of 1 ml/min. A UV detector was set at 250 nm, pressure at 200 kg/ cm2 and absorbance was 0.32 with chart speed 2.5 mm/min. All these parameters were set on HPLC apparatus Shimadzu SPD – 10A VP detector attached with a chart recorder to obtain the chromatogram of the samples.

Samples of Permethrin and Monocrotophos were run on the apparatus. Chromatograms of DDT, Polytrin-C, Deltamethrin, Malathion, Diazinon, Cypermethrin were run under exact same conditions on HPLC. Samples of milk and serum were prepared accordingly and then 20ul of purified sample was injected in the HPLC apparatus. The peaks were compared on the basis of retention time (RT) with the standard peaks. The chromatograms indicated the quantity of pesticide residues in the samples.

The data was statistically analyzed using the formula:

$$T = \frac{\bar{X} - u_o}{\frac{S}{\sqrt{n}}}$$

RESULTS

A total of 40 milk and 6 serum (from breast cancer patients) samples were analyzed. Five serum samples were found to contain organophosphates, sumithion / malathion, while pyretheriod, permethrin was detected in one sample. The serum sample of Zohra Bibi was found to contain sumithion in a concentration of 56.644 µg / 20µl and 65.2972 µg / 20µl, respectively. Serum samples of Tasleem and Saria were found to contain sumithion in a concentration of 92.6293 and 79.6739 µg / 20µl. Serum sample of Najia was found to contain permethrin in a concentration of 50.6494 µg / 20µl (Table 1A).

The analysed milk samples were found to be contaminated with organophosphates, possibly malathion and pyrethriods, permethrin and deltamethrin. Polyttrin-C was detected only in one sample. Malathion was detected in 57.5% of samples while permethrin was detected in 17.5% of samples. The detected levels were higher than the maximum residual limit (MRL) which is 2 ppm for permethrin, 8 ppm for malathion and 1 ppm for deltamethrin. The magnitude of levels higher than the MRL is indicated in Table 1B. Out of 8 milk samples that showed presence of permethrin, 5 samples were collected from Gadap and surroundings while 2 samples were from Kachi Para and Gulshan. The highest level of permethrin i.e., 100 µg/µl was detected in the milk sample of Arzoo from Gadap which was 98 times higher than the MRL (Table 1B). The mean concentration of permethrin was 58.65 µg/20µl. Polytrin-C was detected in the milk sample of Shamsad from Shireen Jinnah colony. Milk samples of Fozia, Farzana, Noor Jehan and Parveen from Karachi showed no pesticide, while milk sample of Hikmat from Gadap also showed no pesticide residue (Table. 1B). Milk sample of Bano Ghaffar from Kemari was found to contain deltametrin and malathion in a concentration of 64.4451 µg/20µl and 18.9312 µg/20µl respectively (Table. 1B).

DISCUSSION

The highest concentration of sumithion was detected in milk sample of Rehmat from Gadap which was 5375 µg/20µl.The milk sample of Hafiza also showed sumithion 99. 5375 µg/10µl while Gul from north Nazimabad showed 99.8888 µg/10µl of malathion. Presence of malathion in more than half of the samples is probably due to the extensive use in Gadap by

Table 1A: Quantification of pesticide residues in serum of breast cancer patients.

Sample no.	Name	R.T.	Pesticide detected	Conc. in µg/20µl
106	Najia	3.007	Permethrin	50.6494
995	Saba	3.189	Malathion	72.4077
107	Tasleem	3.343	Malathion	92.6293
47	Saira	3.322	Malathion	79.6739
998	Kulsoom	3.185	Malathion	65.2972
44	Zohra Bibi	3.478	Malathion	56.6445

Table1B: Quantification of pesticide residues in milk of women residing in Gadap and different areas of Karachi.

Name	Age	Location	Pesticide Detected	R.T.	Conc. In µg/20µl	No. of times higher than MRL.
Khayaloo	35	Mahajir camp	Sumithion	3.316	58.7284	50.7284
Mehboba	30	Gadap	Sumithion	3.252	53.5354	45.5354
Ana Noran	40	Gulgoth	Permethrin	3.048	41.0629	39.0629
Arzoo	27	Gadap	Permethrin	3.020	100	98
Dhani	28	Gadap	Permethrin	3.041	37.2330	35.2330
Nazar	26	Mahajir camp	Sumithion	3.225	85.9060	77.906
Resham	25	Gadap	Sumithion	3.298	99.9113	91.9113
Monis	26	Gadap	Sumithion	3.143	74.9368	66.9368
Asia	30	Zakaria Goth	Permethrin	2.970	98.5946	96.5946
Nazeema	25	New sabzi mandi	Permethrin	3.068	77.4295	75.4295
Hikmat 1	40	Gulgoth	Sumithion	3.267	64.5155	56.5155
Shahzadi	20	Gadap	Permethrin	3.040	64.2696	62.2696
Ghousia	26	Gadap	Sumithion	3.234	97.7971	89.7971
Atiya	21	Gadap	Sumithion	3.234	97.7971	91.6028
Hafiza	26	Gadap	Sumithion	3.240	99.5375	91.5375
Rehmat	25	Gulgoth	Sumithion	3.240	99.5375	91.9785
Bachandi	28	Gadap	Sumithion	3.224	99.0778	91.0778
Mahiyat	27	Gadap	Sumithion	3.197	73.0627	65.0627
Asma	23	Gadap	Sumithion	3.170	99.9589	91.9589
Sakina	29	Sohrab Goth	Malathion	3.125	52.672	44.6728
Bano Ghaffar	30	Kemari	Deltamethrin	2.683	64.4451	63.4451
			Sumithion	3.516	18.9312	10.9312
Shama	26	Gulshan	Permethrin	2.983	39.0845	37.0845
Noor Jehan	26	Gulshan	----------	-------	----------	
Gul	30	N.Nazimabad	Malathion	3.304	99.8888	91.8888
Ayesha	27	Gulshan	Malathion	3.288	79.8140	71.814
Meharunnisa	27	Gulshan	Malathion	3.336	67.6931	59.6931
Chand Bano	33	Gulshan	Malathion	3.192	79.5629	71.5629
Tahira	25	Kachi para, Shireen	Permethrin	2.971	51.4999	49.4999
Urooj	28	Jinnah colong	Malathion	3.161	75.2820	67.282
Fozia	30	N. Nazimabad	----------	-------	----------	
Farkhunda	24	Mehmar camp	----------	-------	----------	
Fozia		Shireen Jinnah				
		colony				
Perveen	25	Masood road	----------	-------	--------	
Shamshad	25	Shireen Jinnah	Polytrin-C			
		colony	a.Prophenophos b. Cypermethrin	a.2.445 b.3.389	a.21.3055 b.73.5890	

farmers and also because it was used in Karachi for 10 years in the antimalarial campaign. Even though malathion is an insecticide of relatively low toxicity, and its presence in the maternal milk is a serious

concern. Same situation is for the presence of permethrin and deltamethrin and Polytrin-C. The p-value of Sumithrion/ Malathion in milk samples was 0.0001 and the p-value for Sumithion/ Malathion in serum samples of breast cancer patients was also 0.0001. Therefore the levels of Sumithion/ Malathion are significantly high. There are several case-control studies reported by some researchers that associate parental exposure to pesticides or pesticide use in the home with childhood brain tumors, lymphomas leukemias, testicular cancers, and in human milk as well as for possible correlation e.g.,. An association of acute nonlymphoblastic leukemia risk has been correlated with pesticide exposure. Association between childhood cancer has also been correlated with the exposure of organophosphate pesticide. The same findings have also been noticed by the present study. Exposure to estrogen throughout a women's life is a risk factor for the development of breast cancer[5]. Similarly Environmental contamination data derived from the breast milk with the exposure of pesticides causes the development of breast cancer[4]. This is almost in line with the findings of the present study. Presence of pesticide residues in human milk like persistent organochlorine, PCDDs, PCDFs and dioxin-like PCBs levels has been reported by many researchers[7,14,15]. The present study also determined the levels of pesticide residues but particularly investigated the levels of organophosphate and pyrethroid residues such as sumithion, malathion, permethrin and deltamethrin in the human breast milk and serum samples of breast cancer patients collected from different areas of Karachi, Pakistan.

CONCLUSION

In view of the analysed milk sample data, it is concluded that the milk samples were found to be contaminated with organophosphate particularly the presence of malathion and pyrethroid residues. It has also been noticed that the most frequently detected pesticides are the malathion / sumithion and the detected levels were higher than the maximum residual limit which is particularly seen in case of malathion. As malathion is an insecticide of relatively low toxicity, so its presence in the mother's milk is alarming. Therefore, through this study it is suggested that the pesticide controlling authority should provide the knowledge to the population about this serious situation.

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Address for Corresponding Author:
Prof. Dr. Muhammad Ahmed Azmi
Department of Physiology, Al-Tibri Medical College
Isra University, Karachi Campus Malir, Karachi
Cell: 0333-2371281
E-Mail: azmiahmed@hotmail.com