

Effect of Pesticide Residues on Health and Different Enzyme Levels in the Milk of Women from Karachi-Pakistan

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Abstract: The aim of this study was to investigate the presence of pesticide residues in human milk and their effects on the enzyme levels (cholinesterase and lactate dehydrogenase) as well as the health status of the pesticide exposed women. Total 135 milk samples from 45 women were collected from nine different divisions of Karachi, Pakistan. In addition ten milk samples were also collected from normal subjects. The milk samples were taken at day 1, day 15 and day 30 from the same women and from the same divisions. The data indicated that only cypermethrin, deltamethrin, malathion and match were identified. The highest concentration 34.86 µg/10 µl of deltamethrin and the lowest concentration 0.336 µg/10 µl of cypermethrin was found in the milk sample. It may be concluded that exposed women showed significant increased and decreased enzyme levels at different division and also complained about the disturbance in the normal functioning of different organ system and possibly produced various ailments and clinically suffered with skin diseases, backache, disturbance in micturition, difficulty in breathing, asthma and hepatitis.

Keywords: Pesticide residues, Human milk, Enzymes, Health.

INTRODUCTION

Pesticides are basically pollutants or mixture of poisonous substances that are found in our environment and used for destroying, controlling and preventing or eliminating different types of pests. The use of pesticides at one side is important in eliminating the pests but on the other side causing risk to the human health when used indiscriminately without knowing their hazardous effects. Because of having poor knowledge and lack of proper guidance most of the persons in Pakistan use synthetic pesticides instead of phytopesticides. As these pesticides reach the human body either directly or via food chain, these are causing various problems like pesticide pollution, resistance and accumulation of pesticide residues in the body of animals and human beings. On this basis the developed countries are preferring the use of phytopesticides and hormonal pesticides under IPM program (Integrated Pest Management) to avoid any harm to human health. Therefore, present study has been carried out to investigate the effect of pesticide residues on health and enzyme pattern in the milk samples of women collected from different divisions of Karachi, Pakistan.

In this connection a study has been conducted and reported that the frequent exposure of pesticide residues such as HCH, DDE and DDT produced hazardous effects in human being [1]. As breast milk is the main and excellent source of nourishment so if mother gets exposed with pesticide, the residues may also be transferred to infants and children through ingestion of maternal milk. Several researchers also determined the presence of organochlorine, 2,3,7,8-TCDD and DDE levels in the breast milk of North American nursing infants and from the two cities of Ukraine [2,3]. The lactational exposure of DDT and HCH in infants as well as the presence of DDT and pyrethroid residues in the breast milk from malaria endemic areas in South Africa was also reported [4,5]. Similarly presence of malathion and OP pesticides in human milk was also reported by some other researchers [6-11]. In Pakistan, pesticide residues were also detected in the human milk from women belonging to the highly polluted areas of Karachi, Pakistan [12].

Many researchers also tried to correlate the different enzymes cholinesterase and lactate dehydrogenase with the harmful effects of pesticides [8, 13-17]. However, very little work has been done in relation to this work in Pakistan [12]. Keeping the hazardous effects in priority, present study has been taken into consideration possibly to find any correlation between the different enzyme levels and the harmful effects of pesticides.

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MATERIALS AND METHODS

Selection of Sampling Sites

Women living in the highly polluted areas affected by the hazardous effects of pesticides and environmental damage by the industrial effluents were selected. Nine different sites of Karachi were selected for the collection of milk samples.

Collection and Storage of Samples

Human milk samples (2-4 ml) were collected three times i.e., at day 1, day 15 and day 30 from the same women and from the same division. Pesticide residues were assayed in their milk samples to observe their effects on enzymes as well as its relation with their health hazards. The milk samples were collected in sterilized glass vials and kept in ice box during collection time. All the samples were then preserved in freezer till analysis.

Quantification of Pesticide Residues

The milk samples were subjected to fat extraction method [18]. The process of sorption was carried out in chromatographic column of alumina and silica [19, 20]. The processed extract was then 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 NH₂), a polar bound phase with particle size of about 7 micrometer in diameter was used. A mobile phase n-Hexane was used with a flow rate of 1ml/min. A UV detector was set at 250 nm, pressure at 200 Kg/ cm² and absorbance was 0.32 with chart speed of 2.5 nm/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 Monocrotophas were run on HPLC apparatus. Chromatograms of DDT, Polytrin-C, Deltamethrin, Malathion, Diazinon, Cypermethrin were run exactly under same conditions on HPLC. Samples of milk were prepared accordingly and then 20 µl of the purified sample was injected in the HPLC apparatus. The peaks were compared on the basis of retention time (RT) with the standard peaks. The area of each peak was calculated to quantify the pesticide residues in the samples.

Statistical Analysis

The data obtained was analyzed statistically by using the Analysis of Variance Technique (ANOVA) and Generalized Linear Model (1988).

Determination of Enzymes

To measure the enzyme activity, kinetic method was used. The enzymatic tests were performed on spectrophotometer UV-160 Shimadzu apparatus. Following enzymes were selected and the quantity was determined in the milk samples of pesticides exposed women.

Cholinesterase

Cholinesterase activity was measured by the calorimetric technique. A kit method from Randox Laboratories (Cat No. CE 190) was used for analysis [21].

Lactate Dehydrpgemase, (LDH)

The activity of lactate dehydrogenase was also measured by using the calorimetric enzymatic kit method [22].

RESULTS

In the present work, a **total 135 human milk samples** were taken from **45 women** and **10 normal control women** for the determination of pesticide residues and enzyme levels. Those which had high level of pesticide residues were taken into consideration for finding the possible correlation between the residual levels and enzyme levels. The different pesticide residues that were detected and quantified in 14 human milk samples from different divisions of Karachi are given in Table 1. The analysis of human milk samples showed that out of **14 milk samples, cypermethrin** was found in noticeable amount in **eight samples, permethrin** was found in **five samples, match** in **one sample** where as **malathion** and **deltamethrin** was found in **two samples** respectively. **Diazinon, polytrin-C, DDT** and **monocrotophos** was **not detected** in any sample. The highest concentration of pesticide i.e., **deltamethrin 34.86 µg / 10 µl** in young lady named **Darakshan** aged 21 years with lactational period of 4 days was noted from **Essa Nagri Colony; cypermethrin 26.8 µg / 10 µl** in **Bi Bi Rehan** aged 20

Table 1: Determination of Pesticide Residue ($\mu\text{g} / 10 \mu\text{l}$) in the Human Breast Milk from Different Divisions of Karachi

Sample Nos.	Division	Match	Cyper.	Delta	Mal.	Perm.
1	Liaquatabad Town	-	-	2.71		-
2.	Liaquatabad Town	-	-	-	12.6	-
3	Liaquatabad Town	-	-	-	17.1	-
4	Essa Nagri Colony	-	26.8	-	-	-
5	Essa Nagri Colony	-	17.31	-	-	-
6	Essa Nagri Colony	-	7.96	-	-	21.8
7	Essa Nagri Colony	-	13.9	34.86	-	-
8	Moosa colony	-	8.94	-	-	-
9	Moosa colony	-	<u>0.336</u>	-	-	12.01
10	Rehri Goth	-	10.2	-	-	-
11	Rehri Goth	-	-	-	-	8.67
12	Rehri Goth	-	-	-	-	22.91
13	Korangi Town	11.21	-	-	-	-
14	Korangi Town	-	8.67	-	-	22.66

Bold= High value, **Underline** = Lower value, - = not detected, **Cyper**= Cypermethrin, **Delta**. = Deltamethrin, **Mal.** = Malathion, **Perm.** = Permethrin.

years with lactation period of 12 days also from **Essa Nagri Colony**; permethrin **22.91 $\mu\text{g} / 10 \mu\text{l}$** in **Hanifa** aged 20 years with lactation period of 6 months from **Rehri Goth** and malathion **17.1 $\mu\text{g} / 10 \mu\text{l}$** was found in **Bi Bi Gul** aged 25 years with lactation period of 2 years from **Liaquatabad division**. The low concentration **0.336 $\mu\text{g} / 10\mu\text{l}$** of cypermethrin was found in the milk samples of **Marium** aged 22 years with lactation period of 10 months from **Moosa colony**, Karachi.

As far as the enzyme levels are concerned **cholinesterase** was found high in **five** subjects and the **highest** quantity **21348.6 U/L (reading – 1)**, **21489.4 μL (reading – II)** and **21888.2 U/L (reading – III)** were noticed in two young female subjects named **Kanza** aged 21 years and **Mahgul** aged 30 years from **Korangi division** respectively. The low concentration of cholinesterase was noticed in 6 female subject from different divisions and the **lowest** quantity **961.86 μL (reading – 1)**, **985.32 U/L (reading – II)** and **1032.24 U/L (reading – III)** was found in the female subject named **Darakhshan** aged 21 years from **Essa Nagri colony** of Karachi city. Only **two female subjects** from **Moosa colony** showed **normal levels of cholinesterase** in all the three timings.

Lactate dehydrogenase (LDH) was found in lower level in seven female subjects e.g., **Bi Bi Hajra (F)** and **Bi Bi Gul (F)** from **Liaquatabad**, **Bi Bi Rehan (F)** from **Essa Nagri colony**, **Najma (F)** and **Marium (F)** from **Moosa colony**, **Gul Andiya (F)** from **Rehri Goth** and

Kanza (F) from **Korangi Town**. The **lowest levels of LDH 857.14 μL (reading – 1)**, **8999.99 μL (reading – II)** and **924.85 U/L (reading – III)** were noted in **two female subjects** named **Bi Bi Rukhsana (F)** and **Hanifa (F)** from the same **Rehri Goth division** of Karachi City. The rest of the milk samples showed normal range of LDH while high levels of LDH was not noted in any samples from any divisions.

DISCUSSION

Women that were highly exposed by pesticide residues indicated that their enzyme levels such as cholinesterase and lactate dehydrogenase (LDH) as well as their health were highly affected. It was noted that from Liaquatabad division one women named Bi Bi Hajra aged 20 years having lactation period of 3 months had high quantity **2.71 $\mu\text{g} / 10 \mu\text{l}$** of deltamethrin in the milk sample. This female subject however showed inhibition of cholinesterase and LDH in all the three readings. The other two subjects from this division named Noor Jahan aged 30 years (F) and Bi Bi Gul aged 25 years (F) both had high quantity **12.6 $\mu\text{g} / 10 \mu\text{l}$** and **17.1 $\mu\text{g} / 10\mu\text{l}$** of malathion. The diagnostic data of Noor Jahan also showed low cholinesterase and normal LDH level where as inhibition of both cholinesterase and LDH was noticed in Bi Bi Gul indicating susceptibility to pesticides. These subjects suffered with hepatitis, liver dysfunction, joint pain, chest pain and also complained about the harmful effects of pesticides on liver function. Similar findings were also reported by other researchers [23, 24].

Table 2: Effect of Pesticide Residues on Health and Enzyme Levels in Effected Women

Divisions	Name Age (Sex)	Lactational Period	Related Health Hazards	Readings	Enzyme Levels (U/L)	
					Cholin	LDH
Liaquatabad	Bi Bi Hajra 20 yr (F)	3 months	Jaundice (3 yrs back) Headache, Backache Joint pain Fever frequently	I	<u>1407.6</u>	<u>1114.282</u>
				II	<u>1446.7</u>	<u>1157.139</u>
				III	<u>1454.5</u>	<u>1199.996</u>
Liaquatabad	Noor Jahan 30 yr (F)	2 months	Chest pain during Hepatitis Liver dysfunction	I	<u>1548.36</u>	2314.278
				II	<u>1571.82</u>	2399.992
				III	<u>1595.28</u>	2442.828
Liaquatabad	Bi Bi Gul 25 yr (F)	1 yr and 6 months	Headache Feel lethargic Jaundice 1 yr back Nausea	I	<u>1431.1</u>	<u>1585.709</u>
				II	<u>1454.5</u>	<u>1628.566</u>
				III	<u>1485.8</u>	<u>1671.423</u>
Essa Nagri Colony	Bi Bi Rehan 20 yr (F)	12 days	No specific illness	I	<u>1219.9</u>	1821.423
				II	<u>1251.2</u>	1842.851
				III	<u>1266.8</u>	1885.708
	Essa Nagri Colony	Maria 20 yr (F)	3 months	Symptoms of pneumonia	I	<u>1571.82</u>
II					<u>1595.28</u>	2357.135
III					<u>1618.74</u>	2399.992
Essa Nagri Colony	Shahida 25 yr (F)	4 months	Respiratory Discomfort Chest pain Hypertension	I	<u>1219.92</u>	1928.565
				II	<u>1243.38</u>	1971.422
				III	<u>1266.84</u>	2041.279
Essa Nagri Colony	Darakshan 21 yr (F)	4 days	Abdominal discomfort Constipation Acidity	I	<u>961.86</u>	1928.429
				II	<u>985.32</u>	1934.327
				III	<u>1032.24</u>	2051.239
Mosa Colony	Nazima 31 yr (F)	21 days	No specific illness complained	I	5442.72	<u>1799.994</u>
				II	5466.18	<u>1842.851</u>
III				5489.64	<u>1885.708</u>	
Mosa Colony	Marium 22 yr (F)	10 months	Jaundice (5-6 months back) General Weakness Feel Sedation	I	6193.44	<u>1371.424</u>
				II	6216.91	<u>1414.281</u>
				III	6240.36	<u>1457.138</u>
Rehri Goth	Gul Andiya 29 yr (F)	10 days	Complained kidney dysfunction Burning sensation in urine	I	15483.6	<u>1028.568</u>
				II	15718.2	<u>1071.425</u>
				III	15952.8	<u>1114.282</u>
Rehri Goth	Bi Bi Rukhsana 20 yr (F)	3 months	Difficulty in breathing Nausea Chest pain	I	14545.2	<u>857.14</u>
				II	14779.8	<u>899.99</u>
				III	15014.4	<u>924.85</u>
Rehri Goth	Hanifa 20 yr (F)	6 months	Nausea Vomiting Joint pain	I	15718.2	<u>857.14</u>
				II	16187.4	<u>899.99</u>
				III	16422.1	<u>924.85</u>
Korangi	Kenza 21 yr (F)	2 yr	Pneumonia Kidney pain Sedation	I	21348.6	<u>1585.709</u>
				II	2189.4	<u>1628.566</u>
				III	21888.2	<u>1671.423</u>
Korangi	Mahgul 30 yr (F)	2 months	Numbness Joint pain Dysprea General Weakness	I	21348.6	2185.707
				II	21489.4	2228.564
				III	21888.2	2271.421

Bold = High value, **Underline** = Low value, Reading I = day 1, Reading II = day 15, Reading III = day 30.

From Essa Nagri colony of Karachi, three young ladies named Maria aged 20 years, Bi Bi Rehan aged 20 years and Shahida aged 25 years had 17.31 µg/10 µl, 26.8 µg /10µl and 7.96 µg /10 µl of cypermethrin respectively, which were found in high levels in these subjects where as another lady from this division named Darakshan aged 21 years also showed high quantity 13.9 µg / 10µl of cypermethrin and a very high quantity 34.86 µg / 10 µl of deltamethrin. All these female subjects showed low levels of cholinesterase in all the three reading except Bi Bi Rehan who also showed low LDH level in the milk samples, while rest of the person had normal LDH level in all the three readings. These persons also complained about the history of respiratory tract infection, chest pain, backache and pneumonia similar to the study reported earlier [25]. Marium aged 22 years (F) from Moosa colony had low level 0.336 µg /10µl of permethrin in the milk sample. This young female subject in addition also showed normal cholinesterase and low LDH levels. Nazima aged 31 years (F) from the same division also showed normal cholinesterase and low LDH probably due the high quantity 8.94 µg/ 10 µl of cypermethrin in the milk sample. This lady also complained about the symptoms of jaundice, general weakness similar to the findings reported by some researchers [26].

In Rehri Goth, Gul Andiya aged 29 years (F) had high level 10.2 µg / 10 µl of cypermethrin. This female subject showed high cholinesterase and low LDH, where as another young lady Hanifa from this division showed very high quantity 22.91 µg / 10 µl of permethrin. On analysis the milk sample of this lady also showed high quantity of cholinesterase and low LDH. Similar findings were also noticed in another female subject Bi Bi Rukhsana aged 20 years also had high quantity 8.67 µg / 10µl of permethrin as well as high cholinesterase and low LDH levels in the milk sample probably due to the direct exposure of this pesticide. All these subjects also complained about the history of kidney dysfunction, burning sensation in urine, difficulty in breathing same to that of previous study [27]. Lastly, Kanza aged 21 years (F) from Korangi division had high level 11.21 µg / µl of match. Beside residues, high cholinesterase and low LDH level was also noticed in this young lady. Another subject Mahgul aged 30 years (F) from this division had high 8.67 µg / 10 µl of cypermethrin and very high quantity 22.66 µg /10 µl of permethrin was noted. In addition she also showed high cholinesterase but normal LDH level in the milk sample besides pesticide residues. These ladies also complained some harmful

signs and symptoms of illness such as pneumonia, nausea, vomiting and also felt chest pain during working. Such findings were also reported by some other researchers [28]. In addition, they also complained the history of sedation, numbness, general weakness same to that of findings reported earlier [29, 30].

Analysis of these findings indicate that the enzymes such as cholinesterase in almost all the subjects from different divisions, the level was high possibly because of exposure of pesticides for long periods, thus resulting in higher tolerance or resistance to the pesticides in these subjects. All the persons who had been living in different divisions for long period get exposed with pesticides seriously and thus suffered with various types of illness. Therefore, due to prolong exposure the level of enzymes have been increased from normal for detoxification of pesticides which may be due to the defect of a particular gene responsible for the synthesis of enzymes. This situation may occur either due to prolonged exposure or indiscriminate use of pesticides in these areas. In case of low or normal cholinesterase found in some persons, the deviation in the level is probably due to lesser exposure of pesticides or may be exposed to sub-lethal or adulterated pesticides.

Correlation between enzymes and pesticides have also been reported by various researchers e.g. The role and significance of enzymes such as lactate dehydrogenase in human milk that is synthesized in the mammary glands produce their actions in response to hormonal stimuli [13]. Another study reported that changes in enzyme activity has been observed in the colostrums, milk and serum samples of mothers and found that milk LDH activity did not change during the first four days of lactation. However, the activity of cholinesterase in the breast milk observed by the present study as compared to the colostrums increased significantly [14]. Similarly, the activity of cholinesterase was found low in the exposed person of Nicaraguan community as compared to control subjects. The present study also noted low cholinesterase level in about 60% of the exposed subjects, thus confirming with the findings of previous study [15]. The quantity of pesticides and their metabolites in the biological samples such as fat, urine, serum and in breast milk was also determined by using analytical technique and biomarkers to detect the effects of pesticides such as cholinesterase inhibition and chromosomal aberration [31]. Similarly, another study reported that the activity of erythrocyte ChE in the pesticide exposed persons

due to OP and carbamates exposure results in the disturbance of enzyme levels due to intoxication. The present study also observed with the findings reported earlier. The activity of cholinesterase was also investigated in the maternal milk of malathion exposed women and found that lactational exposure to malathion causes inhibitory effect of the brain AChE in the offspring [8]. The inhibitory effect of cholinesterase was also noticed by the present study in some cases.

In Pakistan some work has also been done on the enzyme-pesticide correlation. However, such comprehensive work particularly on enzyme pesticide correlation ship has not been done in the human milk randomly on large scale earlier in any particular divisions of provinces in Pakistan. Therefore, the main purpose of this study was to assess the presence of pesticide residues in the breast milk of women and their health risk due to pesticide exposure.

CONCLUSION

In view of the analyzed milk sample data, the pesticide residues and enzymatic activities were investigated in 135 human milk samples from 45 mothers of different divisions of Karachi city. In additionally ten milk samples were also collected from female subjects and considered as normal or control subjects. The data indicated that the activity of cholinesterase was increased significantly where as LDH decreased significantly as compared to control. The most probable reason of this variation in the enzyme level may be due to the involvement of certain factors such as pesticide residues, socioeconomic, metabolic and environmental factors. The pesticides that were detected in the milk samples are cypermethrin, malathion, deltamethrin and match. The most frequently detected pesticides are cypermethrin and permethrin. The sequence of pesticide residues are Cypermethrin > Permethrin > Malathion, Deltamethrin > Match. It is thus concluded that more studies are needed to evaluate the health impact of these consequences in more vulnerable population particularly newborns. Therefore, a surveillance curriculum is desired for children that are exposed to chemical *via* human milk intake as the maternal milk is the excellent source of nourishment particularly for newborns.

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