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Pesticide Residues In Blood Serum Samples from Inhabitants of "Dal Lake" hamlets 'in Jammu & Kashmir, India (2008-2010).

Banday Muddasir[1], Dhar, J K[2], Aslam Shafiqa[3], Qureshi Sabia[4], Jan Tariq[5], Gupta Bhavna[6]

[1, 2, 6] *Indian Institute of Integrative Medicine, Canal, Road Jammu, J&K, India.*
[3] *MM Institute of Medical Sciences & Research , Mullana Ambala, Haryana.*
[4] *Division of Veterinary Public Health, Faculty of Veterinary Sciences ,Shuhama (Aulesteng), Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir. Shalimar campus,191 121.*
[5] *Department of Statistics, University of Kashmir, Hazratbal, Srinagar.*

Abstract: *Dal Lake a Sub Himalyan urban Lake is one of the most beautiful lakes of India and second largest in state of Jammu & Kashmir. The surrounding area of Dal lake and its floating gardens form the most fertile area where a variety of vegetables are grown and extensively consumed by the inhabitants of Dal Lake. An indiscriminate use of wide variety of pesticides have been witnessed to combat pest and increase the yield. The present study was planned to assess the burden of chronic exposure to pesticides by estimation of pesticide residual levels in human serum samples taken randomly from the inhabitants of Dal Lake hamlets. Exposure of humans to hazardous chemicals occurs directly in the fields and indirectly due to consumption of contaminated diet or by inhalation or by dermal contact.The study was conducted from 2008 – 2010 in which a total of 600 blood samples taken from three population groups designated as PG-1 ,PG-2 (Study groups) and PG-3 (Control group). Blood samples were analysed for seven commonly used pesticides viz. Butachlor, γ-HCH, chlorpyrifos, Hexaconazole, Endosulfan 1,Endosulfan 2 and Dichlorovos. Detection and quantification of pesticide residues was performed by Gas Chromatography-Mass spectrometer (Thermo Finnigan Polaris Q type) equipped with Ni Electron Capture Detector. Out of seven pesticides analysed only chlorpyrifos was detected in all the three population groups. Mean concentration of chlorpyrifos in study groups (PG-1 & PG-2) was 0.5194 ± 0.6456 ng/µl and in control group (PG-3) was 0.0008 ± 0.0009 ng/µl. An ANOVA (Analysis of variance) was carried out and a difference was found highly significant in mean concentration of chlorpyrifos between PG-1, PG-2 and PG-3 with F value of 33.39 and P value of 0.000.Tuckey HSD (Honest Significant Difference) test reveals that PG-1 & PG-2 did not differ (P value 0.300) in their mean concentration. PG-3 differs significantly with PG-1 & PG-2 (P value 0.000). This reveals that mean level of pesticide concentration is higher in study population than control group. The findings suggest that chronic low dose exposure to pesticides either directly or indirectly can be a major contributor for presence of pesticide residual levels in human blood.*

Keywords*: Pesticide; chlorpyrifos; Blood; Gas Chromatogram Mass Spectrometer.*

I. Introduction

Pesticides have been used by humans since times immemorial to protect their crops and vectors of human diseases. In spite, of the tremendous uses of pesticides these are also fraught with alarming consequences related to human health and its various components. Rampant and indiscriminate use of pesticides has affected the non target population i.e humans by directly exposing them to serious problems such as teratogenesis, mutagenesis, dermatological, endocrine and neurological dysfunction. Moreover, increased concentration of certain pesticides in the environment are directly related to carcinogenesis. The use of pesticides in large quantities and remarkable biological persistence in environment causes their widespread presence in all elements of food chain, which particularly involves water, vegetation and fish etc. Human beings at the top of food chain, are obviously exposed to harmful effects of pesticides from every quarter. Contaminated diet even with a traces of these hazardous chemicals is a chronic unavoidable source of exposure (Baldassari *et al*.,1995; Harrison *et al*.,1998; John *et al.,* 2001; Bakore *et al*., 2002). Due to their non-polar and lipophilic nature,these are resistant to degradation and persist in the environment and tend to accumulate in lipid rich tissues of the organism (Travis *et al*.,1988) and get biomagnified. So therefore, both human life and environment are equally at a high risk due to noxious effect of these chemicals. (Forget, 1993, Igbedioh, 1991, Jeyaratnam, 1981). Since human health has been a matter of primary concern so many studies which have so far been done arrived at the conclusion that pesticide exposure is associated with chronic health ailments. These primarily include dermatologic, respiratory, memory disorders (Arcury, 2003, O'malley, 1997), neurodeficits (Kamel *et al*., 2003 , Firestone et al., 2005), birth defects and miscarriages (Engel *et al*., 2000, cordes, 1988, Das *et al*., 2001,Eskenazi *et al*., 1999, Garcia, 2003, Moses,1989, Schwartz *et al*., 1986, Stallstones, 2002, Strong *et al*.,

2004,Van Maele Fabry, 2003). Moreover, bioaccumulation of pesticide residues show an association to cause cancer, depression, seizure disorder, liver and kidney dysfunction (Daniels et al., 1997, Sandhu, 1992,Ekbom *et al.,* 1996, Straub et al., 1999, Beseler *et al.*, 2008).

"Dal Lake" one of the most beautiful and famous lakes of India with its pristine glory is second largest lake in state of Jammu and Kashmir. The "Dal" is used for major economic activities relating to tourism, site seeing, recreational activities, fisheries, harvesting of food and fodder plants. The floating gardens of the lake that have originated with time have now assumed a status of biggest vegetable producing bowl of Kashmir. Keeping in view substantial short and long term health risks (WHO-1990) as well as environmental damage/contamination (Conway and Pretty, 1991), associated with indiscriminate use of pesticide a study was designed with an aim to determine pesticide residual levels in blood serum of both occupationally and non occupationally exposed individuals living within and surrounding areas of Dal Lake. It can act as a good indicator to know the quantum of toxic exposure to various pesticides.

II. Materials and Methods

2.1 Collection of sample and population description.

The present study was carried out between the years 2008 and 2010 during which 600 blood samples were obtained from three population groups, with 250 blood samples from population living within Dal Lake designated as PG-1, 250 blood samples from population living 1 km from the shore of Dal Lake designated PG-2 and 100 blood samples from population living more than 4 km away from the Dal Lake designated as PG-3 which acted as control group.(**fig: 1**). All subjects were asked to fill up a questionnaire to gather detailed information about age, sex, ethnicity, marital status, education, occupation, food habits, economic status, health and disease status. With regard to pesticides, a detailed information was acquired, based on predesigned Clinical Record Form (CRF), like occupational use of pesticides, direct or indirect exposure to pesticides, exposure of pesticides through food chain (water, vegetation, fish), Brands /Chemical name of pesticides used, frequency of use of pesticides, seasonality of pesticide use, duration for which pesticides have been used, mode of use of pesticides, quantity of pesticide used, area or crop specific pesticide.

2.2 Extraction of Pesticide Residues in Blood Samples:

Five milliliters of blood from subjects, irrespective of age and sex, were collected in a vaccutainer. All the collected blood samples were centrifuged at 3000 rpm for 20 minutes. After centrifugation, serum was separated and transferred into centrifuge tubes. The extraction process was performed using the method suggested by Bush *et al.,* (1984) with some modifications. All the chemicals used were of analytical grade.

i. To the serum already separated, 5ml of ethyl-acetate and hexane in the ratio of 1:1 was added.
ii. The mixture was shaken vigorously on a vortex mixer for 1 minute.
iii. The mixture was centrifuged for 20 minutes at 3000 rpm.
iv. The organic layer was separated and collected in a round bottom flask.
v. The elute was completely evaporated with the help of a rotary vacuum evaporator . The dried extract was dissolved in 2 ml of ethyl-acetate.
vi. The sample was then ready for analysis by GC-MS/MS.
vii. All compounds were identified by their retention times as compared with known standards.

2.3 Analysis

The analysis includes a qualitative and quantitative estimation of pesticides with the help of Gas Chromatograph Mass Spectrometer (Thermo Finnigan Polaris Q type) equipped with Ni Electron Capture Detector (ECD). The GC operating parameters were as follows: Injector temperature 80 - 270 ^{0}C, first ramp temperature @ 25 ^{0}C/min to 200 ^{0}C, second ramp temperature 2^0 C/min to 230 ^{0}C for 1 min and final temperature @ 20 ^{0}C/min to 280 ^{0}C used for 10 min. Purified helium gas was used as carrier gas and a known volume (25µl) of the sample was injected in. Different peaks of the sample were identified by comparing their retention times with those of standards obtained from pesticide from Pesticides India Limited. Seven commonly used pesticides were chosen and extraction procedures were performed for residual pesticide analysis by GC-MS/MS. The pesticides included were Butachlor (herbicide), Hexachlorocyclohexane γHCH, chlorpyrifos, Hexaconazole Endosufan-I , Endosulfan-2 and Dichlorvos (DDVP).

2.4 Statistical Analysis

Statistical calculation was carried out using the SPSS version 16. Z test was carried out to ascertain any significant difference in mean concentration of detected pesticide between study and control population. A "P" value less than 0.05 was considered to be statistically significant. An ANOVA (Analysis of variance) was carried out by using the SPSS version 16 to verify the difference in mean level of pesticide residual concentration in three groups viz PG-1, PG-2, PG-3 respectively.

III. Results

3.1 Percentage occurrence of pesticide residue.

In the study population (PG-1 and PG-2) 500 blood samples taken randomly from males and females were analysed for selected pesticides. Out of the seven pesticides analysed, chlorpyrifos was the only pesticide detected in 411 (82.2%) of blood samples with 197 (47.9%) being males and 214 (52.1%) being females. In control population (PG -3) 100 blood samples taken randomly from males and females were also analysed for aforementioned selected groups of pesticides. Chlorpyrifos was the only pesticide to be detected in 49 (49%) samples with 26 (53.1%) being males and 23(46.9%) being females. The number and percentage of subjects in study and control population showing presence of chlorpyrifos residual level in blood samples are presented in **Table 1.**

3.2 Concentration of Chlorpyrifos Residual Levels in serum samples with regard to gender, occupational and disease categories.

The mean concentration of chlorpyrifos in the 500 subjects of the study population (PG-1 and PG-2) as shown in Table-2 was (0.5194 ± 0.6456 ng/µl) whereas in control population (PG-3) the mean concentration was (0.0008 ± 0.0009 ng/µl).

The levels of organophosphate pesticide residue viz. chlorpyrifos for all the subjects are shown in Table-3. The mean concentration of this pesticide in the study population in males was (0.5048 ± 0.6475ng/µl) with concentration ranging from undetected to 1.9830 ng/ µl whereas in females it was (0.5326 ± 0.6448 ng/ µl) ranging from undetected to 0.6448 ng/µl. In the control population the mean concentration of chlorpyrifos in males was (0.0008 ± 0.0009 ng/µl) with concentration ranging from undetected to 0.0030ng/µl whereas in females it was (0.0007 ± 0.0008ng/µl) with concentration ranging from undetected to 0.0030 ng/µl.

Comparison of mean concentration of chlorpyrifos level (ng/µl) in males and females, farm workers and non farmworkers, occupational and non-occupational users of pesticides, hypertensives and normotensives, hypothyroid and euthyroid, those with respiratory and no respiratory diseases are presented in Figure:2. Similarly, mean concentration of chlorpyrifos in hypertensive and normotensives, hypothyroids and euthyroids, those with respiratory and without respiratory diseases are presented in figure 3. The figures reveal that females had high level of chlorpyrifos (0.5326 ± 0.6448ng/µl) than males (0.5048 ± 0.6475ng/µl), farmworkers had high level (0.5296 ± 0.6455ng/µl) than non farmworkers (0.5127 ± 0.6466ng/µl), occupational workers of pesticides had high level (0.5213 ± 0.6705ng/µl) than non-occupational workers (0.5185 ± 0.6342) . Likewise hypertensives (0.5763 ± 0.6674ng/µl), hypothyroid (0.5650 ± 0.6324ng/µl) and those with respiratory diseases (0.6517 ± 0.7001ng/µl) had higher levels than normotensives (0.4972 ± 0.6364ng/µl), euthyroids (0.5164 ± 0.6470) and without respiratory disorders (0.4812 ± 0.6195ng/µl).

The highest concentration of chlorpyrifos level was found in individuals with respiratory diseases, followed by hypertensives and hypothyroids respectively.

In control population chlorpyrifos residual level quantified in blood samples was substantially low in all categories of subjects as depicted in Table:3

Comparing mean levels of chlorpyrifos in PG-1, PG-2 and PG-3 it was observed that mean concentration of chlorpyrifos in PG-1, PG-2 and PG-3 was 0.5583 ± 0.6893 ng/µl, 0.4803 ± 0.598 and 0.0007± 0.005 respectively. Moreover, in PG-1 females had higher level (0.5910 ± 0.6890 ng /µl) than males (0.5226 ± 0.0.6886ng/µl). In PG-2, males had slightly higher level (0.4871 ± 0.6060ng/µl) than females (0.4741 ± 0.5933ng/µl) and in PG-3 level of chlorpyrifos in males and females are (0.0008 ± 0.0010ng/µl) and (0.0007 ± 0.0008ng/µl) respectively.

3.3 Concentration of chlorpyrifos in different age groups

While comparing mean concentration of chlorpyrifos in different age groups as shown in Table: 4 it was observed that mean concentration was highest in males (0.5602 ± 0.6985ng/µl) in age group 21- 40 years and among females it was found highest (0.5911 ± 0.6907 ng/µl) in age group 41- 60 years in study population. In occupational workers it was found highest (0.6627 ± 0.7266ng/µl) in age group 21-40 years, in hypertensives it was highest (0.6162 ± 0.7118ng/µl) in age group 21-40 years, in individuals with respiratory disease it was found highest (0.8212 ± 0.8195ng/µl) in 61-80 years age group in the study population. In control population the values are very low in all the categories.

IV. Discussion:

The results of the present study reveals that out of seven pesticide residues analysed only chlorpyrifos (o,o-diethyl-o-(3,5,6-trichloro-2-pyridyl phosphorothioate), an organophosphate pesticide was detected in human serum samples. The results demonstrated an indiscriminate availability and use of organophosphate pesticide chlorpyrifos in vegetable fields. There was also a policy shift towards substituting organochlorines with organophosphates and carbamates, which are considered less persistant and cumulative. Chlorpyrifos is one

of about 100 organophosphate (OP) insecticides on the market today. It is used to kill insect pests by disrupting their nervous system. It is a broad spectrum insecticide used on a wide variety of crop types, for the control of locusts and is present in some cattle dips for the control of ticks and lice. Also registered for domestic gardens, as indoor insect control, termiticide, for pest products and commercial and industrial insect control. Diet is recognized to be a pathway of human exposure to chlorpyrifos yet the relationship between chlorpyrifos residues in food and personal exposure to chlorpyrifos is not well understood (Macintosh et al., 2001). This information would be valuable for evaluating the relationship between personal exposure and possible health effects as low levels of exposure to chlorpyrifos could cause subtle neurological effects (Olson et al.,1998.,Roy et al.,1998) but methods for estimating chlorpyrifos exposure from food intake have not been evaluated.

In the present study chlorpyrifos was detected in 82% of blood samples of study population with concentration mean of (0.5194 ± 0.6456ng / µl) and in control population with a concentration mean of (0.0008±0.009 ± ng/ µl). The higher level of chlorpyrifos in study group than control group can be primarily attributed to the dietary and occupational factors, as the population living within and around the areas of Dal Lake are exclusively dependent on food produced within the Dal Lake in the form of vegetables and fish, as well as dermal exposure by occupational use of pesticides. Some studies suggest that in addition to diet, other potential routes of exposure to chlorpyrifos include inhalation, dermal absorption, and incidental ingestion of soil and settled dust (Whitemore et al.,1994; Zartarian et al., 2000).

Mean concentration of females (0.5326 ± 0.644 ng/µl) was found slightly higher than males (0.5048±0.647 ng/µl). In the valley of Jammu & Kashmir females are aggressively involved in all sort of agricultural practices and males have taken up other occupations besides agribusiness.

Similar results were also observed with some organochlorine pesticide in which ∑DDT concentration was found higher in female populations in Coimbro, Portugal (Lino et al.,2006). Likewise, highest concentration of DDT was found in women from Canary Island in Spain (Zumbido et al., 2005)

It was observed that mean concentration of farmworkers (0.5296 ± 0.6455 ng/µl) and commercial pesticide applicators (0.5213 ± 0.6705ng/µl) were slightly more than non farm workers (0.5127 ± 0.6466 ng/µl) and non occupational users (0.5185 ± 0.6342 ng/µl.) It is quite obvious that both farmworkers and occupational workers of pesticides are directly as well as indirectly exposed to the pesticide application than those not involved directly in agribusiness.

Similar kind of studies were conducted in India in different villages of Punjab (Mathur et al.,2005) in which chlorpyrifos was detected in 85% of blood samples with a mean level of (0.0622 mg/L) and ranged from not detected to 0.4965mg/L. the results correlate with the results obtained in our study.

With regards to study population (SPG1 and SPG2) and control population (SPG3) the difference in the chlorpyrifos residual levels in blood samples between the three groups was determined statistically. An ANOVA (Analysis of Variance) was carried out by using the SPSS Version 16 to establish whether the three groups SPG1, SPG2, and SPG3 differ in their mean chlorpyrifos residual levels. The difference was highly significant with F value of 33.39 and P value of 0.000. Further, Tukey HSD (Honest Significant Difference Test) test was carried out which reveals that SPG1 and SPG2 does not differ (P value 0.300) in their mean concentration. SPG3 differs significantly with SPG1 and SPG2 (P value 0.000). The test reveals that mean level of pesticide contamination is higher in study population than control group. A P-value less than 0.05 was considered to be statistically significant.

Study of health effect was also done in a Dal Lake population which are vulnerable to chronic low dose non-target pesticide exposure. The results reveal that certain diseases in study population are present with a higher percentage than control population. These include hypertension, hypothyroidism, dermatological conditions, respiratory problems, psychiatric disorders and neurological disorders. Chronic low dose exposure of organophosphate pesticide like chlorpyrifos both directly and indirectly can pose a great amount of health risk to the population in long term. Organophosphates are associated with well known acute health problems such as nausea, dizziness, vomiting, headaches, abdominal pain and skin and eye problems.

Organophosphate insecticide exposure has been reported to be associated with affective disorders such as depression, (London et al., 2005). Termite applicators exposed to Chlorpyrifos reported more neurological symptoms including fatigue, loss of muscle strength and depression (Steenland et al, 2000).

Currently the direct estimation of pesticide residual level in biological and environmental samples is a good indicator for assessing the total body burden of pesticide exposure in human and in environment. Alternatively, dietary exposure to chlorpyrifos and estimation of 3,5,6-trichloro-2-pyridinol (TCP y) in urine of healthy volunteers suggests that intake of chlorpyrifos from food is a minor contributor to TCPy in urine (Macintosh et al., 2001).

Studying of health outcomes in a population associated with chronic low level exposure to pesticides is possible by developing some new biological and genetic techniques .The new techniques fall primarily into three areas i) markers of DNA and RNA damage or repair ii) Indicators of oxidative stress and iii) Markers of changes in gene expression related to exposure to pesticide (Bolognese,2003; Toraason et al., 2004). The biomarkers are in a developmental status are have not so far been used in populations extensively exposed to

low dose pesticide and moreover, lack a concrete evidence of an association between the biomarker and specific health outcomes. Nevertheless, they provide potential to increase our understanding of the biological mechanism associated with the health outcomes that have been associated with pesticide exposure in multiple epidemiological investigations.

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