

Effect of Insecticides (Pyrethroids) on Eosinophil Count in School Children

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ABSTRACT

Background: Study was conducted in school children to reveal the toxic effects of pyrethroids which causes allergic reactions with in the body especially altered lung function tests and eosinophil count.

Objective: In the present study, the effects of pyrethroids on eosinophil count of school children were analyzed.

Methodology: The present study is a cross-sectional analytical study. The study was conducted at Government Girls Primary School, Sita Nagar, Karachi including 50 children with ages between 6 – 10 years. They were divided equally into two groups: group A (exposed to insecticidal spray) and group B (exposed to mosquito coil). The correlations between the insecticides (Pyrethroids), and eosinophil by linear regression analysis confirmed that insecticides (Pyrethroids) had a strong correlation with the eosinophil count under study exemplified by the significant increase in Eosinophil count was observed after exposure to mosquito coil whereas there was no association between insecticidal spray and Eosinophil count. Results: There was significant increase in Eosinophil count after exposure to mosquito coil whereas no association was observed between insecticidal spray and Eosinophil count.

Conclusions: The use of spray over the coil is less hazardous to the health.

Keywords: Eosinophil count, Hypersensitivity, Lungs.

Introduction

Pesticides are a category of chemicals that are formulated to kill or repel a pest or halt its reproduction. The naturally occurring pyrethrins are used as a model to produce synthetic pyrethroids from petroleum derivatives. However, synthetic pyrethroids are more persistent in the environment than the naturally occurring pyrethrins and are therefore used indoors as well as in agriculture applications. Pyrethrum is a general name covering both compounds. Pyrethroids affects central and peripheral nervous system of mammals and insects, which cause prolonged opening of Na⁺ channel in the axons. The resulting repetitive action potential evokes hyperactivity, tremor, ataxia, convulsions and possibly paralysis.¹ Pyrethroids are much less toxic than other insecticides in humans. A variety of reversible symptoms such as headache, dizziness, nausea, irritation of the skin and

mucosa, and paresthesia have been reported. They can cause skin rash, upper respiratory tract irritation, allergies and asthma attack.² It has also been documented that exposure to pyrethroids can cause laryngeal symptoms such as hacking, laryngeal pain and hoarseness of voice, coughing, wheezing, difficult breathing and mild obstruction of airways. There have been reports of declining lung functions in some asthmatics due to exposure to insecticide aerosols containing permethrin. Similarly, reduction in lung functions have been observed in pesticide exposed workers. In Agricultural Health Study, permethrin use on animals and crops was associated with wheeze and asthma in farmers.³ There was a report regarding death of an asthmatic girl due to respiratory arrest while using shampoo containing 0.2% pyrethrin. Previous studies in adults showed causative link between

exposure to synthetic pyrethroid and development of motor and sensory disorders including muscle pain, uncertain gait, dizziness, numbness, burning and feeling of pins and needles in the extremities⁵. In children, pesticide exposure was associated with deficiencies in gross and fine motor coordination and short-term memory. Cypermethrin has also been shown to have adverse effects on bone marrow and reproductive system in humans.⁶

The Leucocytes also called the White Blood Cells (WBCs) are the mobile units of the body's protective system. These cells are developed from the multipotent cells in the bone marrow known as hematopoietic stem cell. On the basis of their appearance, they are divided into two subgroups that are Granulocytes and Agranulocytes.⁷ Agranulocytes (mononuclear leukocytes) apparently lack granules and consist of lymphocytes, monocytes, and macrophages. Granulocytes (polymorphonuclear leukocytes) have characteristic cell bound granules when observed under light microscopy. The granulocytes are further divided into subgroups on the basis of their different staining properties namely neutrophils, basophils, and eosinophils.⁸ Eosinophils are bone marrow-derived immune cells that deals with parasites as well as act as inflammatory cells in allergic reactions. These cells also detoxify certain inflammatory substances such as histamine, bradykinin etc released by mast cells and basophils to limit inflammation. The function of Eosinophils is dependent on phospholipase A2 activity.⁹ The development of Eosinophils is primarily controlled by different cytokines (IL-3, GM-CSF, IL-5). Eosinophils actively participates in a number of inflammatory reactions of airways causing elevated levels of eosinophils after the attack of asthma. Eosinophils have special propensity to collect in peribronchial tissues in asthmaticis, parasitic infections, and allergic skin reactions.¹⁰ It is triggered by eosinophil chemotactic factor released by mast cells and basophils and help to kill parasites by releasing different hydrolytic enzymes, reactive forms of oxygen and major basic protein from their granules.¹¹ Eosinophil normally range between 1-3% of peripheral-blood leukocytes and the upper limit of the normal range is 350 cells per cubic millimeter of blood in adults. The normal reference range of eosinophils vary in children according to age, such as at birth it is 0.1-

$2.5 \times 10^9/l$, after 3 days, it is $0.2-2.0 \times 10^9/l$ and at 6 years of life, it is $0.3-0.8 \times 10^9/l$.¹²

Eosinophilia occurs when the number of eosinophils exceeds the normal range and can be arbitrarily classified into mild (351 to 1500 cells per cubic millimeter), moderate (>1500 to 5000 cells per cubic millimeter), or severe (>5000 cells per cubic millimeter).¹³ Sampling blood is the method used for measuring the number of Eosinophils, especially in tissues having mucosal-environmental interface like respiratory, gastrointestinal and lower genitourinary tracts. A high number of eosinophil count is often linked to allergic diseases such as asthma, eczema, hay fever, other causes include autoimmune diseases, leukemia and infections from parasites such as worms.¹⁴ Some drugs that may increase eosinophil count include amphetamines, certain laxatives, certain antibiotics, interferon, tranquilizers. The drugs involved to treat eosinophilia include glucocorticoids, myelosuppressive drugs, and interferon alfa.¹⁵

Methodology

The present study is a cross-sectional analytical study carried out at Physiology Department Fatima Lab, Baqai Medical University Teaching Hospital and Government Primary School, Sita Nagar from July 2013 to December 2013. This study was approved by the Ethical Committee of Baqai Medical University.

Sample size calculation:

$$n = \left[\frac{Z_{\alpha/2} \sigma}{E} \right]^2$$

A total of 50 school children with ages between 6 and 10 years, including boys and girls was taken as test group and was divided equally into two groups A and B. The proforma was filled regarding information about name, age, sex, height (meters) and weight (kg) after taking brief history and consent from parents where necessary. After spraying with Mortein insecticidal spray, the classrooms were then ventilated for 30 minutes and after that, group A students attended their class as per routine. Similarly, in a separate room, group B students were made to sit where Mortein coils were ignited. The process was repeated for three consecutive days. The concentration of pyrethroid

from surface samples was also measured using mass Gas Chromatography from PCSIR Laboratories. Spirometry, lung volumes and airway mechanics can be measured in children. Age, gender, height and weight are measured before the procedure begins.¹⁶

A. Spirometry

Differential Leukocyte Count (DLC)

Collection of Blood:

- 1- The blood was drawn from a vein after cleaning with spirit swab.
- 2- 3 ml of blood was collected in CBC bottle containing EDTA.
- 3- For determining DLC, 1 drop of blood was placed on a glass slide and was spread with another slide by making an angle of 45°.
- 4- Then the smear was allowed to air dry for 2-3 seconds.
- 5- Leishman's stain was poured on each and every slide for 3 minutes.
- 6- Then buffer was poured on the slides for 10 minutes.
- 7- Slide was washed with tap water for 1-2 min and then it was allowed to dry.¹⁷
- 8- The slides were then examined using 40x objective lens starting from the thicker to the thinner end of the film. The eosinophils present were then counted per 100 cells. The percentage of eosinophils was multiplied by the white blood cell count to give the absolute eosinophil count expressed in absolute numbers ($\times 10^9/l$). If one vertical strip does not contain 100 cells, then the net strip may be counted again by going upwards or downwards but not sideways.¹⁸
- 9- The slide was then fixed with 1 drop of DPX.

The eosinophil count was counter checked by Sysmex Auto Analyser.

Determination of Level of Pyrethroid by Gas Chromatography

In present study, commercially available product 'Mortein' in the form of insecticidal spray and mosquito coils were used and applied in accordance with the user's guide i.e. close doors and windows and spray into air for 3-4 seconds. Two coils were ignited in a properly ventilated room having two windows and a door. The 8 samples of

dust were taken from each room after application of spray and coils. The measurements were performed in the month of October with room temperature of 30°C. The active ingredients in Mortein spray and coil were Esbiothrin(d-trans Allethrin) (1.26 g/kg) and Permethrin (0.50 g/kg). The level of detection of Permethrin was 0.01 $\mu\text{g/g}$.

SAMPLE PREPARATION: The samples of dust were taken with the help of swabs dipped in n- hexane about half an hour of application of insecticidal spray and mosquito coil. The samples were kept in air tight plastic bags and refrigerated at 4° C till further analysis. Determination of Permethrin was done by standard accredited method of AOAC, 2007, 18th edition.

Analysis: Quantitative analysis of Permethrin was performed by Perkin Elmer- Clarus 500 Gas Chromatograph using Electron Capture Detector (ECD). Nitrogen was used as carrier and makeup gas and separation was done on DB-5 column. Injector and detector temperature were 270° C and 290° C respectively. Oven temperature programming was 100° C for 15 minutes ramped to 270° C at 20° C/min for 10 minutes.

Results

Group A (Insecticidal Spray): Mean and SEM of Eosinophil count in pre exposure group was found to be 2.64 ± 0.299 , and of post exposure group was 2.84 ± 0.298 . On comparing both groups, non-significant difference ($P > 0.096$) was found.

Group B (Coils): Mean and SEM of Eosinophil Count in pre exposure group was found to be 2.44 ± 0.183 and of post exposure group was 2.88 ± 0.194 . On comparing both groups, significant difference ($P < 0.008$) was found.

| Exposure groups | Mean Eosinophil Count (%) | p-value |
|----------------------|---------------------------|---------|
| Group A (Spray) n=25 | | |
| Pre exposure | 2.64 ± 0.299 | 0.096 |
| Post exposure | 2.84 ± 0.298 | |
| Group B (Coil) n=25 | | |
| Pre exposure | 2.44 ± 0.183 | 0.008 |
| Post exposure | 2.88 ± 0.194 | |

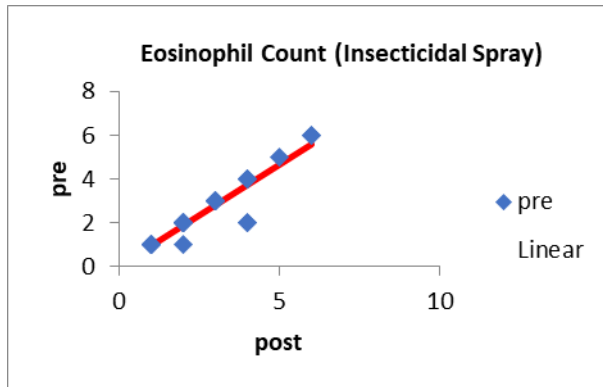


Figure I. Graphical presentation of Correlation of pre and post exposure Eosinophil Count in Group A.

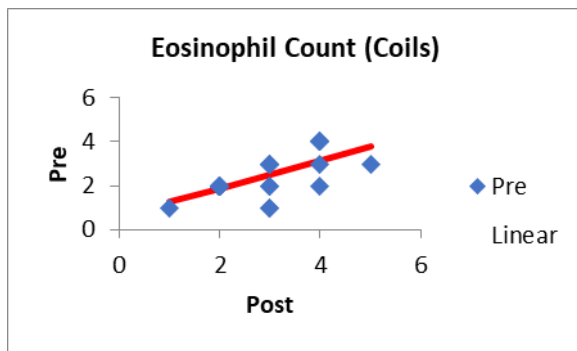


Figure II. Graphical presentation of Correlation of pre and post exposure Eosinophil Count in Group B.

Discussion

There have been reports of itching, burning of the face, nose or throat irritation accompanied by sneezing or coughing in spraymen after spraying with pyrethroid insecticide lambda-cyhalothrin.¹⁹ There is little data available in children concerning the effects of using pyrethroids. Therefore, the present study was carried out to assess the effects of pyrethroids on lung function parameters and eosinophil counts in children aged between 6 to 10 years. The effects of pesticides on total leukocyte count (TLC), red cell count, Hemoglobin content, Hematocrit, MCV, MCH, MCHC and Platelet count have been reported.²⁰

In Pakistan, Naqvi and Jahan (1999)²¹ and Azmi and Naqvi (2005)²² determined the presence of pesticide residues in the blood of Karachi people. In the present study, group A shows the insignificant relationship between insecticidal spray and eosinophil count whereas mosquito coil exposure was associated with an increase in eosinophil count. In one study, the aerosolization of cypermethrin (pyrethroid) was associated with a cough,

fever, dyspnea, wheezing, blood eosinophilia and interstitial lung infiltration in 110 cases.²³ Similar study was conducted in mice to reveal the toxic effect of cypermethrin which caused systemic hypersensitivity predominantly in lungs via eosinophils and neutrophils.²⁴⁻²⁵ In our study, the insecticidal spray was not associated with change in eosinophil count whereas significant increase was observed in eosinophil count after exposure to mosquito coils

Conclusion

The correlations between the insecticides (Pyrethroids) and Eosinophil count by linear regression analysis confirmed that insecticides (Pyrethroids) and Eosinophil counts had a strong correlation under study. There was a significant increase in Eosinophil count after exposure to mosquito coil whereas no association was observed between insecticidal spray and Eosinophil count. The use of spray over the coil is less hazardous to the health.

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