Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10799258

EVALUATION OF SERUM TESTOSTERONE ALTERATIONS ON EXPOSURES TO PYRETHROID PESTICIDE IN MALE FARMERS FROM HYDERABAD, SINDH, PAKISTAN

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Abstract

The purpose of this study was to determine the relationship between pesticide exposure and circulation levels of male reproductive hormones in agricultural community. To assess variations in serum testosterone level, eighty (80) male spray workers/farmers were participated and exposed to the parathyroid lambda-cyhalothrin. A comprehensive questionnaire was used to collect extensive information about recent, collective lifetime use of pesticides and other agricultural- related activities. Participants' exposure to pesticide, protective tools, and health status were also assessed over the study period. Blood samples were sought before and after applications of pesticides to evaluate the serum testosterone levels. Results were assessed statistically. When compared to their pre-exposure levels 5.77±0.98ng/ml, it was recorded that sixty (60%) of the spray workers had significantly lower ($P < 0.05$) serum testosterone level 4.77±0.28 ng. /ml. Out of these 60%, (21%) spray workers had highly significant ($P < 0.01$) declined, serum testosterone level (2.79%±0.40ng/ml) was below normal. Dizziness, headaches, joint pain, nausea, and allergies to the skin and eyes were noted as clinical symptoms linked to the pesticide exposure. It has been shown that the pesticide lambda cyhalothrin may disrupt the hypothalamic-pituitary- testicular axis, which can reduce serum testosterone production and potentially harm spray workers' reproductive systems.

Keywords: Pesticides; Lambda-Cyhalothrin; Parathyroid; Spray worker; Testosterone.

INTRODUCTION

In order to protect agriculture crops from hazardous insects and to enhance production, different pesticides and agrochemicals are increasingly being applied in agriculture fields around the world, especially in developing countries like Pakistan. The Pesticides can be used safely and properly; nevertheless, if they are used indiscriminately, excessively, or without consideration for the environment or human health, serious issues may result.

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This not only damage crops and economic pests, but also other living organisms and their populations in agricultural fields exposed to pesticides [1, 2].

As an androgenic hormone, testosterone is essential for the male reproductive system, including spermatogenesis, normal sexual function and shaping of genital morphology [3]. In addition, testosterone controls the immunological and body composition systems [4]. Inadequate levels of serum testosterone have been associated to impaired sexual function, body mass, and muscle size [5], as well as inhibit spermatogenesis [6]. The alteration of testosterone may also be responsible by exposure to endocrine-disrupting chemicals (EDCs) [7].

Male infertility has drawn more attention globally during the past few decades [8]. Evidence of the decreasing sperm concentration in males from Europe and Africa has been studied during the past 50 years [9, 10]. Male infertility could be attributed to a variety of factors. The concentration of sperm may be declining for a variety of reasons, including alcohol, endocrine disruptors, and pesticides exposure. Research has been carried out on the notorious toxic effects caused on exposure to endocrine disruptors to the human body in everyday life such as organic pollutants, heavy metals, and pesticides.

Male infertility affects over 30 million men globally, making about 40% to 50% of all instances of infertility [11, 12]. Men who are infertile usually have a great psychological load that includes anxiety, sadness, trauma, stress, embarrassment, inadequacy, and personal suffering, in addition to societal issues including discrimination, relegation and divorce [13, 14]. Infertility in males has significant negative effects on the economy, but it is also becoming more well recognized as a sign of general health issues in men [15]. Nevertheless, most of the infertility research that is now accessible focuses on infertility in women. As a consequence, little is understood about the male aspects of infertility. A significant class of pesticides with several household and agricultural uses, pyrethroids are used as insecticides. And are regarded as possible EDCs [16, 17]. And [18] Pyrethroid use has dramatically expanded during the last several decades on a global scale. The major causes of the rising pyrethroid consumption are their excellent insecticidal properties, delayed pest resistance, and wide spectrum nature. In addition, there are further benefits of pyrethroid intake, including less environmental persistence, quicker metabolism, less human toxicity because of restricted skin absorption, and fewer tissue accumulation [19]

A thorough review of the literature on epidemiology revealed a connection between male infertility and pyrethroid exposure [20]. As an example, pyrethroid exposure has been linked to male reproductive toxicity. Additionally, concerns have been raised about the quality of semen, sperm DNA, reproductive hormones, pregnancy outcomes, and developmental abnormalities [21] Poor semen quality has been shown in other studies, with men exposed to pyrethroids are showing lower sperm count and aberrant sperm morphology [22].

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As a result, the current research sought to investigate whether the pyrethroid pesticide ingredient may interfere with the development of the reproductive system by impeding serum testosterone levels.

This was because the literature on lambda-cyhalothrin pesticide is lacking. The results of this study should either give the scientific community the information needed to plan future research on the relationships between pesticide exposure and changes in reproductive hormones, or they should assist the country's public health system in developing efficient preventative measures and interventions to protect farmers' health.

MATERIALS AND METHODS

Study Design and Assessment of Pesticide Exposure

The experimental study was conducted in agricultural fields of districts Hyderabad, Sindh, Pakistan particularly cotton growing areas. These were extremely pesticide-intensive areas where crops were grown whole year.

Eighty (80) male spray workers, 20 to 50 years old. All spray workers/farmers had a professional connection to agricultural fields. Before spraying insecticide exposure, a questionnaire was used to gather information on socio-demographics, medical and occupational history, pesticide usage, lifestyle, and reproductive history of farmers.

Five milliliters (05 ml.) of blood were then drawn from each spray worker during 6:00 am to 7:00 am. Participants responded to a brief questionnaire on recent pesticide usage and other exposures linked to agriculture at the second visit during harvest season. Male farmers and occupational spray workers gave their agreement before taking part in the research. They were asked if they had handled pesticide containers or had not come into direct contact with pesticides in the previous few days.

Using a lever-operated sprayer tank, the pesticide was applied to the crops for a continuous three hours. Following three days of pesticide exposure once again, five milliliters (05 ml.) of blood were drawn in the early morning from every exposed spray worker.

For every spray worker, scores of safety precautions, protective measures and symptoms were noted. A rating system ranging from 1 to 5 was devised for safety and protective tools and a proportion of symptoms linked to exposure was also collected for every spray worker. Data on every spray worker was later transferred to Performa.

Test Chemical

The insecticide Lambda-Cahalothrin (2.5 % EC), pyrethroid synthetic insecticide, was used. The test chemical was made in accordance with the recommendations of the Sindh Agriculture Department, Government of Sindh, Sindh Agriculture University, Tando Jam, Sindh, Pakistan. The approved quantity for cotton was lambda-cyhalothrin 2.5% EC 330 ml per acre.

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Serum Testosterone Analysis

Both blood samples before exposure and after pesticide exposure of farmers labeled properly and maintained in an ice box samples were kept at -20 °C on dry ice to laboratories. Blood samples that had been collected were transported to the Liaquat University of Medical and Health Sciences' research and diagnostic laboratory in Hyderabad, Sindh, Pakistan, so that serum testosterone levels could be likened to the anti-androgenic action of lambda-cyhalothrin. These blood samples were subjected to a biochemical evaluation using the Electrochemiluminescent (ECL) technological procedure using an immunoassay analyzer from a contemporary laboratory system, the Cobas e411. The collective term for this method is Electochemiluminescene Immunoassay (ECLIA).

Statistical Analysis

Using the Microsoft Office 2016 data base on the Windows 10 version, all of the data was produced and computerized. For data scoring chart, a simple algorithm was created. Information provided as Mean ± SD using the Student's t test method [23]. The P value of less than 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

The aim of the present research was to assess the correlations and deviations in their serum testosterone levels after being exposed to pyrethroid pesticides among male farmers who worked in the agricultural fields of Hyderabad, Sindh, Pakistan. The investigation was conducted in regions that cultivate cotton. The detected quantities of serum testosterone represent recent exposures since the serum indicators examined in this investigation were acquired both before and after exposure to pesticides.

The Participants' General Characteristics

Eighty (80) men spray workers, ages 20 to 50, participated in the research. 67 (84%) of the participants were spray workers who used frequently agrochemicals/pesticides for farming, and 13 (16%) were males who infrequently used pesticides or agrochemicals at work. A summary of the study population's general characteristics is provided. The spray workers and farmers had variable experiences in using, preparing, and applying pesticides and agrochemicals, but they were not aware of any reliable sources for advice on managing pesticide substances. The farmers and spray workers often lack the necessary knowledge and experience regarding the use of pesticides and agrochemicals, despite official prohibitions on using insecticides [24] Furthermore, the farmers routinely disposed of empty pesticide containers in the agricultural fields and poured residual, diluted pesticide solutions on the ground. The improper disposal of empty containers and careless handling of agrochemicals and their residues were also indicative of farmers' ignorance of the danger of pesticides and might potentially contribute to pollution of the environment [25]. Thus, it is inevitable that humans and other non-targeted animals would come into contact with these dangerous chemical residues [26]. The current research

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also showed that, spray workers were not using the proper safety precautions or protective equipment while spraying. Only a few spray workers, dressed appropriately in boots and glasses, had a piece of cloth covering their face and nose. Every spray worker's score on safety and protective measures for pesticide exposure was recorded. Compared to the maximum score of five (05), 20% of spray workers had the highest score of 03. Additionally, 40% of spray workers received no credit for their safety precautions (Fig. 1).

1. Clinical signs of pesticide exposure during and after spraying included headaches, dizziness, backaches, sore joints, nausea after spray tears, irritations to the skin and eyes, blurred vision, sweating, and breathing difficulties, which were common in the majority of spray workers (Fig. 2).
2. According to the findings of the interview reports on the general and reproductive health conditions of the farmers and spray workers, sexual weakness and exhaustion were the most often reported reproductive issues, whereas premature ejaculation was the least common. Users of pesticides often reported more symptoms related to reproductive dysfunction.

Evaluation of the Testosterone Profile

In males, testosterone affects every organ and system and has a significant impact on every aspect of the body, including behavior, mental health, sexuality, skills, and social standing [27]. These characteristics might be altered by changes in serum testosterone levels. Furthermore, a drop in blood testosterone levels may result in a reduction in spermatogenesis, infertility, or sterility [28]. The relationships between pesticide exposure and blood testosterone levels were examined in this research. Serum testosterone levels are correlated with Leydig's cell production capacity. Changes in blood testosterone levels might be a sign of pesticide exposure-induced inhibition of the enzymes involved in testosterone production or exposure-induced malfunction of Leydig's cells. Testosterone regulates the release of Gn, RH, and LH from the anterior pituitary gland and the brain via a negative feedback system, which in turn influences spermatogenesis [29, 30].

In the current investigation, compared to previous pesticide exposure, 60% of spray workers and farmers had a significant ($P < 0.05$) change in blood testosterone levels after exposure to lambda- cyhalothrin (Table 1 and Fig 3). Following exposure, their mean blood testosterone level was 4.77±0.28 ng/ml. But before exposure, it was 5.77±0.98 ng/ml. It has been discovered that pesticide exposure impairs reproductive function by changing reproductive hormone levels or causing oxidative stress [31, 32]. Similarly, exposure to pyrethroids has been shown to have negative impacts on reproductive health in other animal investigations. For instance, harmful effects on the male reproductive system were shown by a comprehensive review and meta-analysis on pyrethroid exposure in rats [33]. Out of the 60% of participants in the current research who had a significant change in their serum testosterone level (21%) had blood serum testosterone levels below normal by 2.79±0.40ng/ml, which indicated extremely significant ($P < 0.01$)

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changes in their serum testosterone level (Table 2). This demonstrates a disturbance in the pituitary-testicular axis and adverse consequences on the male gonads after pesticide exposure. However, 40% of farmers and spray workers had non-significant changes in their blood testosterone levels (Table 3).

Moreover, pyrethroid exposure has been linked to decreased testosterone levels in rat experiments. Furthermore, the weight of the testicles and epididymis showed a link [34]. A research conducted on farmers in the agricultural area of southern Brazil found that the usage of lambda-cyhalothrin recently was linked to hormone levels in the male population, raising concerns regarding reproductive health (Santos et al., 2019). [35] It was shown in several previous studies that there is a negative correlation between pyrethroid metabolites and the quality of semen, which includes a reduction in sperm count, abnormal sperm cell shape, and lower testosterone levels in men [36].

Overall, present study's findings indicated that the pyrethroid insecticide lambda-cyhalothein may interfere with or change blood testosterone levels, which may then have an impact on male reproductive processes and result in infertility.

RECOGNITIONS / ACKNOWLEDGMENTS

The National Centre of Excellence in Analytical Chemistry (NCEAC), University of Sindh, Jamshoro, Pakistan, and Prof. Dr. Kazi Suleman Memon, Sindh Agriculture University, Tando Jam, Pakistan are greatly appreciated by the authors for their insightful and helpful scholarly advice during this study. They also thank the participants, spray workers/farmers, and agriculture land lords.

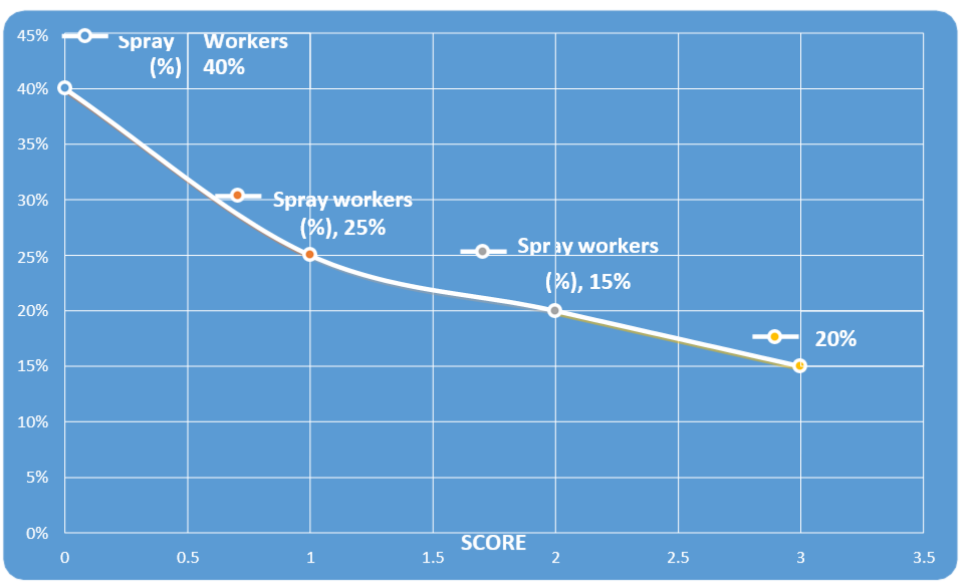

Figure 1: Showing Observed Score of Safty / Protective Measures In Spray Workers during Spraying. The Score was considered by Scoring Range 1-5 and Zero was recoded for No Protective Tools

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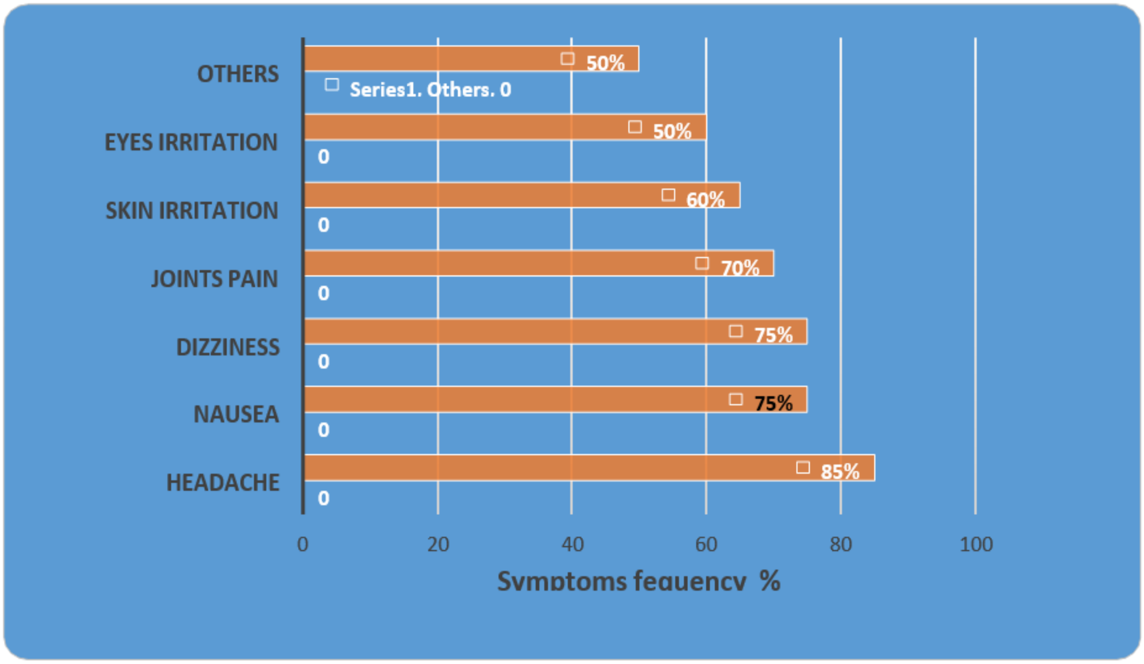

Figure 2: Showing, Symptom's Frequency and Numbers in Spray Workers Exposed to Lambda-Cyhalthrin Pesticide during Study

Table 1: Showing Significant Alterations in Serum Testosterone in Spray Workers after Exposure to Lambda Cyhalothrin

No. of spray workers (Total) n =48/80	Normal serum levels Testosterone (Rang) ng. /ml. (20-70 years)	Optimal Anti-Aging range of serum Testosterone ng. /ml. (20-70years)	Values of Testosterone before spraying ng. /ml. (Mean ±SD)	Values of Testosterone after Spraying ng. /ml. (Mean ±SD)	Effect in (%)
48	2.83-12	5.00-8.00	5.77±0.98	4.77±0.28*	60% (P < 0.05)

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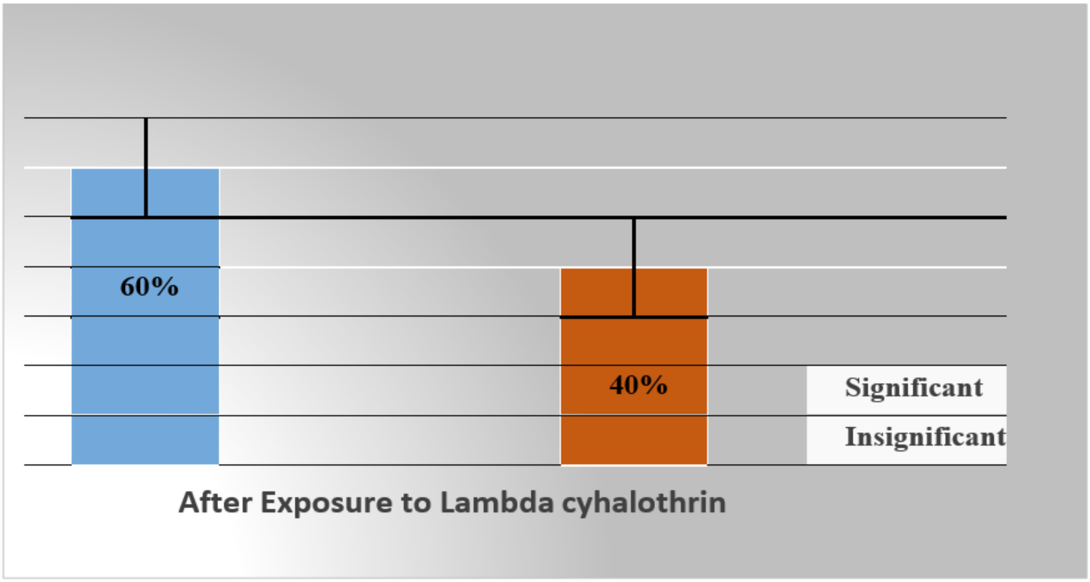

Figure 3: Showing Percentage (%) with Significant, Alteration and Insignificant Alterations in Serum Testosterone in Spray Workers after Exposure to Lambda Cyhalothrin

Table 2: Showing Highly Significant Alterations Serum Testosterone Level Out of (60%) Spray Workers with Significance Change after Exposure to Lambda Cyhalothrin Pesticides

No. of spray workers (Total) n =10/48	Normal serum levels Testosterone (Rang) ng. /ml. (20-70 years)	Optimal Anti-Aging range of serum Testosterone ng. /ml. (20-70years)	Values of Testosterone before spraying ng. /ml. (Mean ±SD)	Values of Testosterone after Spraying ng. /ml. (Mean ±SD)	Effect in (%)
10	2.83-12	5.00-8.00	5.77±0.98	2.79±0.40**	20% ($P < 0.01$)

Table 3: Showing Spray Workers with Insignificant Alterations in Serum Testosterone Directly even after Exposure to Lambda Cyhalothrin Pesticides

No. of spray workers (Total) n =32/80	Normal serum levels Testosterone (Rang) ng. /ml. (20-70 years)	Optimal Anti-Aging range of serum Testosterone ng. /ml. (20-70years)	Values of Testosterone before spraying ng. /ml. (Mean ±SD)	Values of Testosterone after Spraying ng. /ml. (Mean ±SD)	Effect in (%)
32	2.83-12	5.00-8.00	5.39±0.89	5.27±0.84	40% ($P > 0.05$)

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