BioScientific Review (BSR)
Volume 7 Issue 3, 2025
ISSN$_{(P)}$: 2663-4198, ISSN$_{(E)}$: 2663-4201
Homepage: https://journals.umt.edu.pk/index.php/bsr

Title:	**Evaluating Cardiovascular Risk: Lipid Profile Changes Due to Pesticide Exposure in Agricultural Sprayers - A Case-Control Study**
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DOI:	https://doi.org/10.32350/bsr.73.02
History:	Received: November 29, 2024, Revised: May 16, 2025, Accepted: June 19, 2025, Published: July 21, 2025
Citation:	Nawaz A, Maqsood K, Liaqat F, Nizam N, Roohi N. Evaluating cardiovascular risk: lipid profile changes due to pesticide exposure in agricultural sprayers - a case-control study. *BioSci Rev.* 2025;7(3):10–19. https://doi.org/10.32350/bsr.73.02
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Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Evaluating Cardiovascular Risk: Lipid Profile Changes Due to Pesticide Exposure in Agricultural Sprayers - A Case-Control Study

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ABSTRACT

Introduction. There are several threats to the human body from the potentially hazardous chemicals used in crop fields, such as pesticides. This investigation aims to assess variations in the serum lipid profile associated with pesticide exposure.

Methodology. For this case-control study, 131 participants were recruited. These included 71 pesticide sprayers and 60 healthy individuals who served as the control group, all from the rural areas of South Punjab, Pakistan. The lipid biomarkers of the participants were evaluated using a biochemistry analyzer and the results were statistically analyzed through GraphPad Prism.

Results. The results indicated that TG (triglycerides) and TC (total cholesterol) levels significantly ($p<0.001$) increased with a prominent increase in LDL-C (low-density lipoprotein cholesterol) and VLDL-C (very low-density lipoprotein cholesterol) ($p<0.001$ and 0.011 respectively). However, a significant decrease in HDL-C (high-density lipoprotein cholesterol) ($p=0.006$) was observed in sprayers as compared to the control group.

Conclusion. These alterations may be attributed to oxidative stress and hepatic dysfunction induced by prolonged pesticide exposure. The results are based on the hypothesis that significant alterations in the lipid profile anticipate potential health risks associated with pesticide exposure.

Keywords: cardiovascular risk, lipids, pesticides, sprayers

1. INTRODUCTION

Pesticides include a wide variety of chemicals, increasingly being used all over the world and commonly used in agriculture, to enhance crop production and control pests [1]. These chemical substances are known for their toxic properties which, when exposed to certain concentrations, can have detrimental effects on various forms of life. These compounds can infiltrate the human body, disrupt metabolic processes, and interfere with the normal functioning of enzymes [2]. Tons of synthetic pesticides are deposited to increase the yield of crops. This scenario poses a significant threat to non-target organisms because 98% of sprayed pesticides directly or indirectly affect them. Several studies estimated that 80% of sprayed pesticides directly contaminate the environment [3].

Farmers use synthetic pesticides to protect their crops against pest infestations. However, the elevated utilization of these synthetic chemicals is a growing concern.

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Moreover, issues arise due to the inadequate precautions taken by pesticide applicators [4]. The substances used to eliminate pests can also pose risks to animals and beneficial species including earthworms, which play a crucial role in enhancing soil health [5].

Approximately 2 million tonnes of pesticides are utilized annually worldwide. China is the major contributing country, followed by the USA and Argentina. In 2022, the total amount of pesticides used in agriculture was 3.70 million tonnes, representing a 4% increase from 2021, a 13% increase over a decade, and a doubling since 1990 [6].

Exposure to pesticides has been linked to a range of health issues, including hypersensitivity, allergies, hormone disruption, cancer, and asthma, among other diseases [7]. Pesticide subjection has additionally been connected with birth abnormalities, reduced birth weight, fetal mortality, and various adverse consequences [8]. Furthermore, exposure to pesticides, whether unintentional or intentional, can lead to fatalities [9]. The residues of pesticides have been detected in human breast milk samples, raising concerns about potential exposure during pregnancy and the well-being of newborns [10]. Hence, the increase in pesticide consumption has a negative health impact. Studies point to an association between exposure to pesticides and cardiovascular diseases (CVDs), one of the leading causes of mortality around the world [11].

Lipids are integral constituents of living membranes and are significant in synthesizing various hormones [12]. High-density lipoprotein (HDL) mitigates the chances of arteriosclerosis with various distinct mechanisms [13]. Additionally, plasma triglycerides ratio to HDL-C and LDL-C also act as fundamental and primary indicators in predicting ischemic heart disease (IHD) [14].

In individuals with intermediate risk, low-density lipoprotein (LDL) serves as an indicator for assessing the risk of coronary health, rather than being used for CVD treatment. Furthermore, HDL cholesterol and the combined ratios of HDL-C and LDL-C are subjects of ongoing investigation in the context of cardiovascular risk [15].

Different studies have been conducted worldwide about health issues due to pesticide exposure, although limited data is available about the cardiovascular risk posed due to this type of exposure. Thus, it was hypothesized that exposure to pesticides induces cardiovascular risk in pesticide sprayers. So, the current study aimed to investigate the risk of cardiovascular disorders due to the detrimental effects of pesticides among the sprayers of South Punjab, Pakistan.

2. METHODOLOGY

The ethical review board of the Institute of Zoology, University of the Punjab, Lahore approved the current case-control study plan. For this study, many agricultural farms of different crops (sugarcane, cotton, wheat, fruits and vegetables) situated in the rural areas of South Punjab, Pakistan were visited. Pesticide sprayers (n=71) were recruited as the study group. As the control group, healthy subjects (n=60) of the same age groups and from the same areas were selected. The predominant crops in the region are cotton and wheat. The participants were primarily involved in spraying pesticides on these crops. Commonly used pesticides in the region include chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, imidacloprid, glypho-

sate, and metsulfuron-methyl. A questionnaire was designed and distributed before blood sampling to record the participants' demographic and medical details and to assess their height, age, weight, drug addiction, professional skills, and occupational and medical reports. During recruitment, informed consent was taken in writing from all subjects.

A certified technician was engaged for blood sampling and all precautionary measures were followed. Blood samples (5cc) were taken in the morning after 12 hours of fasting. Blood was poured into the coagulant activator vial for 30 minutes at 25°C. The samples were centrifuged at a speed of 3000rpm at a local clinical lab setup for serum separation within two hours of collection. The serum was then separated into labelled Eppendorf and carried to the Physiology Lab, Institute of Zoology, University of the Punjab, Lahore in an ice box. Further, it was stored at -80ºC for biochemical analysis.

For this study, serum lipid profiles (TG, TC, HDL-C, LDL-C, VLDL-C) of sprayers and control group were measured by using commercially available kits of Monlab, Spain using photometer (5010) v5plus, ROBERT RIELE, Germani. The analysis was conducted in the Physiology and Endocrinology Laboratory, located within the Institute of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. Statistical analysis was done in GraphPad Prism (version 5.01) of the obtained data by applying t-test with a significance level of $p<0.05$. The results were reported as mean ± SEM in both tabular and graphical forms.

3. RESULTS

Among the exposed group, most participants were 18-50 years old, had normal body weight, and were married. Sprayers had been exposed to pesticide for a period of 5-25 years (Table 1). Among the lipid profile parameters, triglyceride (TG) levels showed a significant increase ($p < 0.001$) with 25% elevation in the exposed group. Total cholesterol (TC) was also markedly elevated ($p < 0.001$) by 14%. While, high-density lipoprotein cholesterol (HDL-C) decreased significantly ($p = 0.006$) by 16% in the exposed group, as compared to controls. On the other hand, low-density lipoprotein cholesterol (LDL-C) increased significantly ($p < 0.001$) by 23% and very low-density lipoprotein cholesterol (VLDL-C) was also significantly elevated ($p = 0.011$) by 19% in sprayers, as compared to healthy controls (Table 2, Figure 1 a-e).

Table 1. Sociodemographic Characters of Study Subjects

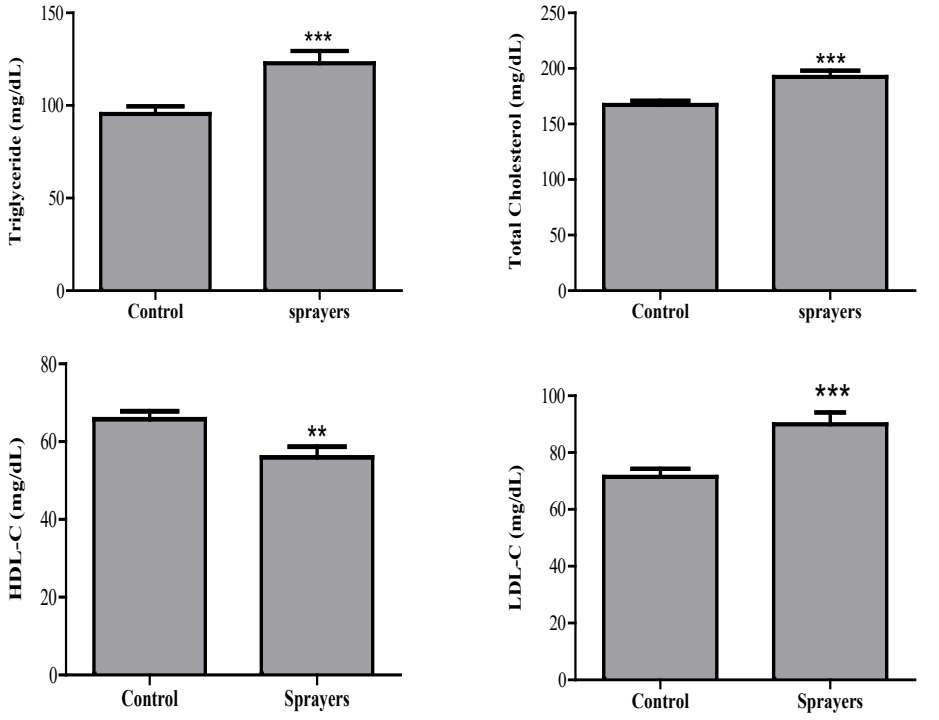
Parameters	Category	Control (*N*=60)		Sprayers (*N*=71)	
		N	%	*N*	%
Age (Years)	<20	9	15.00	11	15.49
	20–30	21	35.00	21	29.58
	31–40	19	31.67	20	28.17
	>40	11	18.33	19	26.76
BMI (kg/m^2)	Underweight	8	13.33	9	12.68
	Normal weight	39	65.00	44	61.97
	Overweight	8	13.33	11	15.49
	Obesity	5	8.33	7	9.86
Marital status	Married	35	58.33	54	76.06
	Unmarried	25	41.67	17	23.94

Parameters	Category	Control (*N*=60)		Sprayers (*N*=71)	
		N	%	*N*	%
Education	Illiterate	13	21.67	32	45.07
	Primary	18	30.00	28	39.44
	Secondary	17	28.33	9	12.68
	Higher	12	20.00	2	2.82
Exposure (Years)	<10	0	0.00	36	50.70
	10-21	0	0.00	23	32.39
	>21y	0	0.00	12	16.90

Table 2. Comparison of Lipid Profile in Control and Pesticides Sprayer Group

Parameters	Mean ± SEM		*p*-value	Percentage difference
	Control (60)	Sprayers (71)		
TG (mg/dL)	95.48 ± 4.04	122.80 ± 6.60	<0.001	25↑***
TC (mg/dL)	167.10 ± 3.70	192.30 ± 5.69	<0.001	14↑***
HDL-C (g/dL)	65.75 ± 2.09	55.95 ± 2.72	0.006	16↓**
LDL-C (mg/dL)	71.42 ± 2.91	89.93 ±4.22	<0.001	23↑***
VLDL-C (mg/dL)	20.55 ± 1.10	24.99 ± 1.28	0.011	19↑*

Note. *, ** and *** represent significance at $p < 0.05$, 0.01 and 0.001, respectively
↓ : Decrease, **↑:** Increase



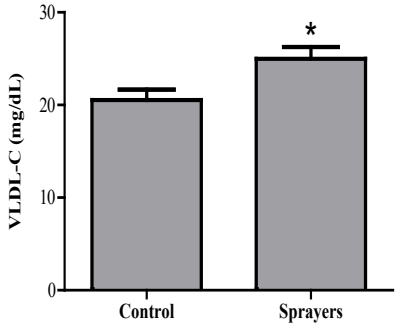


Figure 1. Comparison of serum level of TG, TC, HDL-C, LDL-C and VLDL-C (mg/dL) in sprayers with comparison to control group. Values are Mean ± SEM. TG; triglycerides, TC; Total cholesterol, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol and VLDL-C**;** very low-density lipoprotein cholesterol.
*, **, *** indicate Significance at p <0.05, 0.01 and 0.001, respectively.
↑; Increase, ↓; Decrease.

4. DISCUSSION

In Pakistan, approximately 70% of the population resides in rural areas, with a significant portion of the labor force directly or indirectly exposed to pesticides used in the agricultural sector [16]. Pesticides are chemicals widely employed to combat various undesirable pests or organisms afflicting the crops. As mentioned previously, pesticides can have far-reaching ecological consequences and can cause damage to multiple body organs due to their potent biological activity and, at times, their prolonged presence in the environment [17]. Compared to the worldwide average, Pakistan has a high consumption of pesticides, with an alarming increase of 1169% during the last two decades [18].

As reported earlier, the increase in lipid biomarkers indicates a noteworthy alteration in hepatocyte permeability. The results of this research were corroborated by another investigation involving Tunisian farmers subjected to pesticides over a long duration. These farmers demonstrated a prominent lipid metabolism disruption. These disruptions can contribute to the development of circulatory problems including atherosclerosis and cardiac pathologies. Importantly, lipid abnormalities in individuals with type 2 diabetes play an important part in the progression of atherosclerosis [19].

The findings of this research suggest that groups exposed to pesticides exhibit elevated cholesterol levels, possibly because of prolonged exposure to these chemicals. The obstruction of the bile duct within the liver, leading to a reduction or interruption in the secretion of cholesterol in the first part of the small intestine, could contribute to the observed fluctuations in TC levels [20]. Elevated TC levels have been associated with more threats of coronary artery diseases and atherosclerotic diseases, along with a range of body organ-related health issues [21].

The current research aligns with a study conducted by Setiawan, which demonstrated that a significant factor, namely hyperlipidemia, contributes to atherosclerosis and cardiovascular development. The development of dyslipidemia over time, in turn, increases the risk of coronary diseases in workers. This is particularly concerning because the higher total cholesterol or LDL-C range in the bloodstream is a pivotal threat of chronic artery disease (CAD) [22].

The current study found that the average TG levels in sprayers exposed to pesticides exceeded those of the control group.

Consequently, there was a substantial increase in TG levels (16.98%), as compared to the control group. As proposed by Freeman in 1993, the metabolism of triglycerides is controlled by the LPL enzymes. Pollutants also influence LPL enzymes as LPL hydrolyzes triglycerides and removes them from circulation at the tissue level [23].

In the current study, it was observed that HDL-C levels decrease among sprayers exposed to pesticides when compared to the control group. Framingham study, conducted in the 1980s, also highlighted the elevated levels of HDL-C, specifically ranging over 30-60 mg/dl, as significantly lowering down the risk of coronary disease in individuals. Several studies have indicated an inverse correlation between HDL-C and coronary heart disease. Consequently, HDL-C is included among the risk factors that healthcare professionals utilize to assess the likelihood of developing coronary heart disease [24].

HDL cholesterol is considered as a significant factor in developing novel therapeutic approaches to prevent cardiovascular diseases. Moreover, the VLDL-C levels were notably raised in the group exposed to pesticides, signaling a substantial cardiac risk associated with pesticide exposure in individuals.

This finding reveals insignificant and noteworthy variations in the lipid profile (including LDL-C, HDL-C, VLDL-C, TG, and TC) which can potentially contribute to various health issues in human beings, such as coronary heart disease and atherosclerosis [25].

4.1. Conclusion

The study reveals a significant correlation between pesticide residues and alterations in lipid biochemical markers, suggesting that these biomarkers could be useful in evaluating the health impact of pesticides on farmworkers. The risk of developing cardiovascular disorders rises when proper precautions, such as using protective gear, ability to read pesticide labels, and safely disposing of toxic materials, are lacking. Without effective measures to reduce pesticide exposure at work, a considerable number of individuals may face cardiovascular issues at a younger age.

AUTHOR CONTRIBUTION

Alina Nawaz: data curation, writing –original draft. **Kaleem Maqsood:** conceptualization, writing –review & editing. **Farwa Liaqat:** data analysis. **Naira Nizam:** writing –review & editing. **Nabila Roohi:** conceptualization, resources, supervision.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

FUNDING DETAILS

No funding has been received for this research.

GENERATIVE AI DISCLOSURE STATEMENT

The authors did not used any type of generative artificial intelligence software for this research.

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