

Original Research Article

Bivariate and multivariate regression analysis of pesticide exposure duration, protective practices and PON1 (192Q/R) polymorphism among agricultural workers in Malerkotla and Rupnagar Districts of Punjab, India

Vinay Kumar*, Rajdeep Kaur

Department of Medical Lab Technology and Biotechnology, RIMT University, Mandi Gobindgarh, Punjab, India

Received: 18 April 2026

Revised: 15 May 2026

Accepted: 19 June 2026

*Correspondence:

Dr. Vinay Kumar,

E-mail: vinaykumar30583@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Occupational pesticide exposure poses a significant public health risk among agricultural workers, particularly in developing regions with limited safety practices. Genetic variability, especially in the paraoxonase 1 (PON1) gene, influences individual susceptibility to organophosphate toxicity. The PON1 (192Q/R) polymorphism affects enzyme activity involved in pesticide detoxification.

Methods: A cross-sectional study was conducted at RIMT university hospital among 200 pesticide-exposed agricultural workers from the Malerkotla and Rupnagar districts of Punjab, India. Participants (184 males, 16 females) were selected based on occupational exposure. Exposure characteristics and safety practices were assessed using structured questionnaires. Genotyping of the PON1 (192Q/R) polymorphism was performed using PCR-RFLP. Bivariate logistic regression was used to examine associations between genotype and gender, while multivariate logistic regression evaluated relationships between exposure variables using combined genotype (QR+RR vs QQ) and allele (R vs Q) models. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results: No significant association was observed between PON1 polymorphism and gender. The QR genotype showed higher odds of occurrence in females (OR=3.61; $p=0.28$), whereas the RR genotype showed reduced odds (OR=0.70; $p=0.52$). The R allele was not significantly associated with gender (OR=0.75; $p=0.59$). Multivariate analysis indicated a gradual increase in the odds of R allele-containing genotypes with longer pesticide exposure, with workers exposed for >40 years showing higher odds (adjusted OR \approx 2.08), though not statistically significant ($p=0.08$). Protective measures were associated with reduced odds but lacked significance.

Conclusions: PON1 (192Q/R) polymorphism was not associated with gender; however, prolonged pesticide exposure showed a non-significant trend toward increased R allele prevalence, highlighting the need for improved occupational safety practices.

Keywords: 192Q/R. Gene-environment interaction, Agricultural workers, Multivariate logistic regression, Occupational health, Personal protective equipment, Pesticide exposure, PON1 polymorphism

INTRODUCTION

Modern agriculture makes extensive use of pesticides to control pests and increase crop yield, but their widespread

and frequently uncontrolled use poses serious health risks to people, especially agricultural workers.¹ Numerous negative health consequences, such as neurotoxicity, endocrine disruption, respiratory disorders and

carcinogenic effects, have been linked to long-term pesticide exposure.² Because of their strong acetylcholinesterase inhibitory effects and widespread use in developing nations, organophosphates are a particularly concerning class of pesticides.³ According to field data and district-level records, farmers in Punjab's Rupnagar and Malerkotla districts use a variety of pesticides from various chemical classes. The most commonly reported pesticides are organophosphates.⁴ Pesticide exposure at work mostly happens through skin contact, inhalation and unintentional ingestion.

In low- and middle-income countries, inadequate training, a lack of awareness and limited access to personal protective equipment (PPE) all increase the risk of exposure.⁵ Poor adherence to safety procedures, such as failing to wear gloves, masks and protective clothes, as well as disregarding label instructions when handling pesticides, has been repeatedly documented in studies.¹ Genetic susceptibility has an additional impact on the health effects of pesticide exposure. The detoxification of organophosphate pesticides is largely dependent on the paraoxonase 1 (PON1) enzyme.³ Polymorphisms in the PON1 gene impact the efficiency and activity of the enzyme. The 192Q/R variant, which modifies enzymatic activity by substituting arginine (R) for glutamine (Q), is one of the most extensively researched polymorphisms.⁶ The ability of individuals with different genotypes (QQ, QR and RR) to metabolise harmful pesticide compounds varies, which affects their vulnerability to negative health effects.⁷

Recent research indicates that PON1 polymorphisms and pesticide exposure may interact genetically to affect health outcomes differently in exposed populations.⁸ However, little information is available from agricultural communities in Punjab, India's developing districts of Malerkotla and Rupnagar, where exposure levels are frequently high and safety procedures are inadequate. Furthermore, multivariate analytical methods have not sufficiently examined the combined impact of genetic variability, occupational practices and exposure duration.

Therefore, using both bivariate and multivariate regression analysis, the current study investigates the duration of pesticide exposure, protective practices and PON1 (192Q/R) polymorphism among agricultural workers in the districts of Malerkotla and Rupnagar in Punjab, India.

METHODS

This cross-sectional study included 200 agricultural workers from the Malerkotla and Rupnagar districts of Punjab, India, who were mostly exposed to organophosphate pesticides. Based on their exposure to pesticides at work, male participants (n=184) and female participants (n=16) were selected for the study. The study was conducted at RIMT University Hospital from September 2022 to June 2025. Participants were chosen from nearby rural communities and agricultural fields

using convenience sampling. Participants had to be at least eighteen years old and have been exposed to pesticides for at least a year. Individuals who refused to give consent, had genetic disorders or had chronic systemic illnesses were excluded.

A structured questionnaire was used to gather data via in-person interviews. Data on demographics, occupational exposure and safety protocols were collected. Exposure-related factors included the duration of pesticide use, the type of agricultural field, the method of pesticide application and post-application hygiene practices. Among the safety practices assessed were the use of personal protective equipment, such as gloves, boots and eye protection, as well as adhering to label instructions and understanding pesticide handling guidelines. The RIMT University Institutional Review Board (IRB) Committee in Mandi Gobindgarh, Punjab, approved the current study (IRB/RIMT/2022/02). A structured questionnaire was created in English and translated into the study area's native languages, Punjabi and Hindi, to make sure participants could understand it.

Standard phenol-chloroform techniques or commercial kits were used to extract genomic DNA in accordance with established protocols.⁹ Spectrophotometric analysis was used to evaluate the concentration and quality of DNA. A PCR-RFLP method was used to analyse the PON1 (192Q/R) polymorphism. For the codon Q192R polymorphism, the DNA fragments were amplified using the forward 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and reverse 5'-CCT GAG AAT CTG AGT AAA TCC ACT-3' primers.

A final volume of 25 µl was used for each PCR reaction, which contained 100 ng of genomic DNA, 400 nM of each primer, 200 µM of each dNTP (NEB), 10 mM Tris HCl, 50 mM KCl, 1.5 mM MgCl₂ (pH 8.4) (NEB) and 1.5 U Taq polymerase (AmpliTaq). 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds are followed by the initial denaturation at 94°C for five minutes. final extension for seven minutes at 72°C. AlwI was used to digest the PCR products, which produced 66 and 172 bp fragments for the 192R allele and an undigested 238 bp fragment for the 192Q allele.¹⁰ For genotype identification, 3% agarose gel electrophoresis was used to resolve amplified PCR and digested restriction enzyme PCR.

The Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA) was used to conduct the statistical analysis. The Hardy-Weinberg formula was used to determine allele frequencies, while the gene counting method was used to determine genotype frequencies. The genotype and allele frequencies of the two groups of people were compared using the chi-square test. The odds ratios (ORs) and 95% confidence intervals (CIs) for the relationship between PON1 polymorphism and gender were calculated using bivariate logistic regression analysis. The following relationships between

exposure variables were assessed using multivariate logistic regression analysis: Allele model (R vs. Q allele); combined genotype model (QR+RR vs. QQ). 95% confidence intervals (CIs) for adjusted odds ratios (ORs) were computed. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

The exposure characteristics and safety practices of 200 pesticide-exposed workers 16 females and 184 males were examined. All participants (100%) were engaged in open-field farming activities. The duration of pesticide exposure was highest in the 10–20 years category (31.0%), followed by 1–10 years (27.5%) and 20–30 years (26.0%). The 30–40 year old (8.5%) and >40 year old (7.0%) groups showed smaller percentages (Table 3).

There was very little use of personal protective equipment (PPE). When applying pesticides, none of the workers reported wearing eye protection, reading the labels or following the directions. Just 6.0% wore gloves and 1.0% wore protective shoes. While 42.5% used mechanical methods, the majority (57.5%) applied pesticides with their bare hands. 96.0% of workers reported washing their hands after using pesticides, indicating comparatively better hand hygiene practices.

The distribution of PON1 (192Q/R) genotypes showed that the QQ genotype was the most prevalent in both females (68.8%) and males (64.7%), followed by the RR genotype (25.0% in females vs. 33.7% in males), while the QR genotype was least frequent (6.2% vs. 1.6%).

The overall genotype frequencies were 65.0% (QQ), 2.0% (QR) and 33.0% (RR). Statistical analysis using the chi-square test revealed no significant difference in genotype distribution between genders ($\chi^2=1.946$, $df=2$, $p=0.378$) (Table 1 and Figure 1).

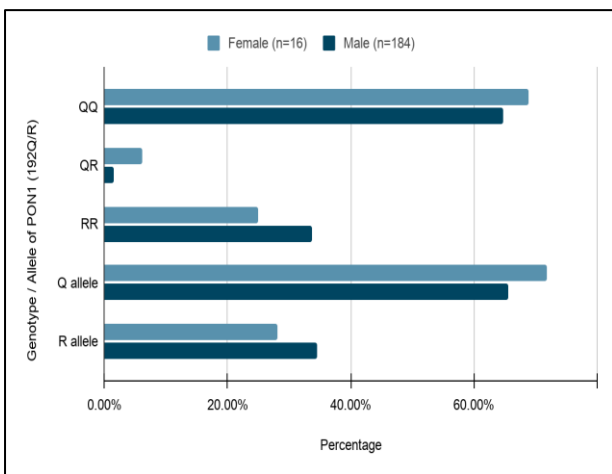


Figure 1: Distribution of genotypes and alleles of PON1 (192Q/R) by sex or gender in pesticide-exposed workers (n=200).

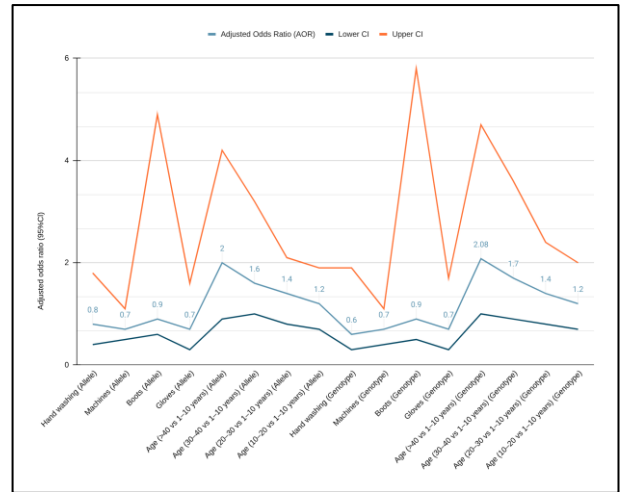


Figure 2: The line plot showing adjusted odds ratios (OR) with 95% confidence intervals for factors associated with PON1 (192Q/R) polymorphism using combined genotype model (QR+RR vs QQ) and allele model (R vs Q) among pesticide-exposed workers.

The frequency of the Q allele was higher in females (71.9%) compared to males (65.5%), whereas the R allele was more frequent in males (34.5%) than females (28.1%). However, this difference was not statistically significant ($\chi^2=0.288$, $df=1$, $p=0.591$).

Bivariate logistic regression was performed to evaluate the association between PON1 (192Q/R) polymorphism and gender, using QQ genotype as the reference. Individuals with the QR genotype had higher odds of being female compared to QQ (OR=3.61; 95% CI: 0.34–37.7; $p=0.28$).

The RR genotype showed lower odds of association with female gender (OR=0.70; 95% CI: 0.21–2.27; $p=0.52$). Allelic analysis further showed that the R allele was not significantly associated with gender (OR=0.75; 95% CI: 0.34–1.67; $p=0.59$) (Table 2). Overall, no statistically significant association was observed between PON1 (192Q/R) polymorphism and gender. Multivariate logistic regression analysis using the combined genotype model (QR+RR vs QQ) demonstrated that increasing duration of pesticide exposure was associated with a gradual rise in the odds of carrying R-allele-containing genotypes. Workers with more than 40 years of exposure showed higher odds (adjusted OR \approx 2.08), although the association did not reach statistical significance ($p=0.08$).

Similarly, the allele model (R vs Q) revealed a consistent trend of increased R-allele frequency with prolonged exposure. Protective practices, including the use of gloves, boots and machine-based pesticide application, were associated with reduced odds of R allele carriage; however, these associations were not statistically significant ($p>0.05$) (Figure 2). These findings indicate a trend toward increased genetic susceptibility with longer exposure duration, although definitive associations could not be established.

Table 1: Distribution of genotypes and alleles of PON1 (192Q/R) by sex or gender in pesticide-exposed workers (n=200).

Gene	Genotype/Allele	Female (n=16)	Male (n=184)	Total	χ^2 (df)	P value
PON1 (192Q/R)	QQ	11 (68.8%)	119 (64.7%)	130 (65.0%)	1.946 (2)	0.378
	QR	1 (6.2%)	3 (1.6%)	4 (2.0%)		
	RR	4 (25.0%)	62 (33.7%)	66 (33.0%)		
	Q allele	23 (71.9%)	241 (65.5%)	264 (66.0%)	0.288 (1)	0.591
	R allele	9 (28.1%)		136 (34.0%)		

*Significant at p<0.05

Table 2: Bivariate logistic regression for PON1 (192Q/R) and sex or gender in pesticide-exposed workers (n=200).

Genotype	OR	95% CI	P value
QQ (Ref)	1	—	—
QR vs QQ	3.61	0.34 – 37.7	0.28
RR vs QQ	0.7	0.21 – 2.27	0.52
R vs Q allele	0.75	0.34 – 1.67	0.59

Table 3: Exposure data and safety procedures followed by the pesticide-exposed agricultural workers (n=200).

Exposure data/safety practice	Categories	Pesticide-exposed workers (n=200)	(%)
Type of field	Open fields	200	100.0
Years of pesticide use/exposure	1–10	55	27.5
	10–20	62	31.0
	20–30	52	26.0
	30–40	17	8.5
	>40	14	7.0
Wear an eye mask	Yes	0	0.0
	No	200	100.0
Wear special shoes/boots	Yes	2	1.0
	No	198	99.0
Wear special gloves	Yes	12	6.0
	No	188	94.0
Method of application	Bare hands	115	57.5
	Machines	85	42.5
Wash hands after application	Yes (water wash/oil wash)	192	96.0
	No	8	4.0
Read labels on pesticide containers	Yes	0	0.0
	No	200	100.0
Follow the product label	Yes	0	0.0
	No	200	100.0

DISCUSSION

The present study investigated the association between the PON1 Q192R genetic polymorphism and gender among pesticide-exposed workers and found no statistically significant relationship. Both chi-square analysis and logistic regression consistently demonstrated that genotype and allele distributions were comparable between male and female participants, indicating that gender alone does not influence the distribution of the PON1 Q192R polymorphism in this exposed population. Reports from South Asian and other non-European

populations are consistent with the QQ genotype's predominance found in this study. Research on Indian and neighbouring populations has shown that the Q allele is more common than the R allele, especially in rural and occupationally exposed groups.^{11,12} These results emphasise the significance of taking geographic and ethnic variation into account when interpreting PON1 genotype distributions because significant inter-population differences have been well-documented. Logistic regression analysis revealed that female participants had higher odds of having the QR genotype, but this association was not statistically significant and came with

a large confidence interval, indicating the estimate's imprecision. This is probably because there weren't many female participants in the study. Similar findings have been documented in environmental and occupational genetic studies, where small subgroup sizes, especially among women, lead to erratic odds ratios with no biological significance.^{12,13} Although this result was not statistically significant, the RR genotype demonstrated lower odds in females, indicating a relative predominance among male workers. Previous experimental and epidemiological studies have shown that the R allele has higher catalytic efficiency for certain organophosphate oxons, whereas the Q allele is less efficient for the detoxification of several substrates.^{14,15}

Despite these functional differences, there is still conflicting evidence regarding the distribution of gender-specific genotypes and the majority of research indicates that physiological factors and exposure intensity have a greater impact on the differences in enzyme activity between men and women than genotype frequency alone. Allelic analysis in the present study further confirmed the absence of gender-based association, with the R allele showing no significant effect. This result is consistent with reports suggesting that PON1 polymorphisms are more important for susceptibility and health outcomes after exposure than for demographic stratification like age or sex.^{15,16}

A consistent trend of higher odds of R-allele carriage with longer duration of pesticide exposure was noted, even though the multivariate logistic regression analysis did not show statistically significant associations. The R allele was more likely to be carried by workers who had been exposed for more than 40 years, indicating a potential cumulative effect of long-term exposure. Similar findings have been reported in studies looking into gene-environment interactions, where prolonged exposure may influence genetic susceptibility or selection patterns.^{7,17} The lack of statistical significance in the current study could be attributed to the small sample size in higher exposure categories and the low variability in protective practices.

The protective trends associated with the use of gloves, boots and machine-based pesticide application emphasise the significance of safe handling practices in reducing occupational exposure. Previous research has shown that wearing appropriate PPE significantly reduces dermal and inhalation pesticide exposure, lowering associated health risks.¹ Nevertheless, a significant gap in occupational safety compliance is highlighted by the low prevalence of PPE usage in the current study, which makes it difficult to establish strong statistical associations.

The reliability of the observed trends was reinforced by the allele-based analysis's results, which agreed with the genotype model. This internal consistency suggests that the relationship between pesticide exposure and PON1 polymorphism, although not statistically significant, may

have biological relevance and warrants further investigation. Furthermore, the study's cross-sectional nature limits causal inference and longitudinal studies are required to better understand the temporal relationship between exposure and genetic outcomes.

The study was limited by its relatively small sample size and cross-sectional design, which restricted causal interpretation of the findings. No detailed quantitative assessment of pesticide exposure or serum paraoxonase enzyme activity was carried out. Furthermore, environmental and lifestyle-related confounding factors were not fully assessed. To validate these results, more extensive longitudinal studies that include biochemical and molecular evaluations are needed.

CONCLUSION

The findings suggest that the PON1 (192Q/R) polymorphism is not associated with gender among pesticide-exposed workers. The study provides evidence of a potential association between prolonged pesticide exposure and increased prevalence of R allele-containing genotypes, along with alarmingly poor safety practices among agricultural workers. These findings emphasise the need for targeted interventions, including education, training and policy enforcement, to promote safe pesticide handling and reduce occupational health risks.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

- Romero Damalas CA, Koutroubas SD. Farmers' exposure to pesticides: Toxicity types and ways of prevention. *Toxics*. 2016;4(1):19.
- Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. *Sci Total Environ*. 2017;575:525–35.
- Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE. Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. *Toxicology*. 2013;307:115–22.
- Devika D, Aulakh RK, Sharma R, Rani N. Analysis of pesticide and heavy metal bioaccumulation in earthworms from Central plain zone of Punjab. 2024.
- Remoundou K, Brennan M, Sacchetti G, Panzone L. Factors affecting pesticide exposure in agriculture: A review of the literature. *Int J Environ Res Public Health*. 2014;11(7):6952–89.
- Furlong CE, Holland N, Richter RJ, Bradman A, Ho A, Eskenazi B. PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity. *Pharmacogenet Genomics*. 2006;16(3):183–90.
- Lee BW, London L, Paulauskis J, Myers J, Christiani DC. Association between human paraoxonase gene

- polymorphism and chronic symptoms in pesticide-exposed workers. *J Occup Environ Med.* 2003;45(2):118–22.
8. Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, et al. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. *Neurotoxicology.* 2013;39:158–68.
 9. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: A laboratory manual.* 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 1989.
 10. Gupta A, Saluja A, Saraswathy KN, Imnameren L, Yadav S, Dhamija RK. PON1 (Paraoxonase 1) Q192R gene polymorphism in ischemic stroke among North Indian population. *Ann Indian Acad Neurol.* 2022;25(1):100–105.
 11. Mitra S, Kshatriya GK. Genetic variation at Q192R and L55M polymorphisms in paraoxonase-1 (PON1) among Indian populations. *Environ Toxicol Pharmacol.* 2016;45:182–90.
 12. Jaiswar SP, Priyadarshini A, Singh A, Ahmad MK, Deo S, Sankhwar P. Association between PON1 (L55M and Q192R) genetic polymorphisms and recurrent pregnancy loss in North Indian women exposed to pesticides. *Rev Bras Ginecol Obstet.* 2021;43(11):805–10.
 13. Srídon A, Ruchiwit K, Suwannahong K, Pongstaporn W, Sudjaroen Y. PON1 Q192R gene polymorphism and pesticide exposure status of rice farmers. *Indian J Forensic Med Toxicol.* 2020;14(4):3051–7.
 14. Costa LG, Richter RJ, Li WF, Cole T, Guizzetti M, Furlong CE. Paraoxonase (PON1) as a biomarker of susceptibility for organophosphate toxicity. *Biomarkers.* 2003;8(1):1–12.
 15. Costa LG, Furlong CE. Paraoxonase (PON1) in health and disease: Basic and clinical aspects. *Crit Rev Toxicol.* 2012;42(9):633–53.
 16. Andersen HR, Wohlfahrt-Veje C, Dalgård C, Christiansen L, Main KM, Nellemann C, et al. Paraoxonase 1 polymorphism and prenatal pesticide exposure associated with adverse cardiovascular risk profiles at school age. *PLoS One.* 2012;7(5):36830.
 17. Singh S, Kumar V, Thakur S, Banerjee BD. DNA damage and oxidative stress in pesticide-exposed workers: Role of genetic polymorphisms. *Toxicol Appl Pharmacol.* 2011;252(2):114–22.

Cite this article as: Kumar V, Kaur R. Bivariate and multivariate regression analysis of pesticide exposure duration, protective practices and PON1 (192Q/R) polymorphism among agricultural workers in Malerkotla and Rupnagar Districts of Punjab, India. *Int J Res Med Sci* 2026;14:2833-8.