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Original Article

Serum Levels of Circulating Immune Complexes and Nitric Oxide in Farmers Exposed to Pesticides in Southwestern Nigeria

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Abstract: The widespread use of pesticides in agriculture has raised significant concerns about their long-term health effects, particularly among chronically exposed farmworkers. This study investigated the serum levels of circulating immune complexes (CICs) and nitric oxide (NO) in pesticide-exposed farmers in Southwestern Nigeria, exploring their role as biomarkers of immune activation and oxidative stress. A case-control study design was employed involving 50 farmers with ≥10 years of pesticide exposure and 39 unexposed controls. CIC and NO levels were quantified using polyethylene glycol precipitation and Griess reagent assays, respectively. Data were analyzed using Mann-Whitney U and Kruskal-Wallis tests. Correlations were evaluated using Spearman's rho. Farmworkers demonstrated significantly higher CIC levels (87.21 ± 60.34 Eq/ml) compared to controls (60.16 ± 40.74 Eq/ml; $p < 0.05$). NO levels were also markedly elevated in exposed farmers (88.74 ± 35.92 µM) relative to controls (53.41 ± 22.79 µM; $p < 0.05$). A significant positive correlation was found between NO levels and duration of pesticide exposure ($r = 0.489$, $p < 0.001$), while a negative correlation existed between CIC levels and exposure duration ($r = –0.367$, $p < 0.05$). Chronic pesticide exposure is associated with heightened immune complex accumulation and oxidative stress, predisposing farmworkers to immune dysregulation and potential long-term health risks. Regular biomonitoring using CIC and NO may offer early insight into pesticide-induced immunotoxicity.

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INTRODUCTION

The global demand for increased agricultural productivity has led to extensive and sustained use of synthetic pesticides, which remain central to modern crop protection practices. These agrochemicals are employed to control pests, weeds, and plant diseases, thereby enhancing yield and economic gain. However, the growing dependence on pesticides, particularly in low- and middle-income countries (LMICs), has raised serious environmental and public-health concerns. Recent data from the Food and Agriculture Organization (FAO, 2024) reveal that more than 3.7 million tonnes of active pesticide ingredients were applied worldwide in 2022—an increase compared with preceding years—with developing regions, including sub-Saharan Africa, accounting for a rising proportion of this use. Weak regulatory enforcement, limited farmer education, and poor monitoring systems contribute to unsafe handling and chronic exposure (FAO, 2024; Boedeker et al., 2020).

Nigeria, one of the leading agricultural producers in West Africa, has about 70 % of its rural population directly engaged in farming. Organophosphates, carbamates, and pyrethroids are the most frequently used pesticide classes, while organochlorines such as DDT, aldrin, and chlordane have been officially banned under national law and the Stockholm Convention (FMEnv, 2021). Nonetheless, legacy environmental contamination and occasional illegal use of these persistent organic pollutants remain documented across Nigerian agro-ecosystems (Gbaruko et al., 2009; Pimentel et al., 2024). Several local investigations have reported low awareness of safe-use practices and inconsistent application of personal protective equipment among smallholder farmers (Oguntade et al., 2020; Yusuf et al., 2022; Adebayo et al., 2023). Such deficiencies increase the likelihood of chronic low-dose pesticide absorption, resulting in neurotoxic, reproductive, endocrine, carcinogenic, and immunotoxic effects (Mokarizadeh et al., 2015; Dhouib et al., 2016).

Among the recognized biomarkers of immunological and oxidative stress in pesticide-exposed populations are circulating immune complexes (CICs) and nitric oxide (NO). CICs are antigen–antibody aggregates normally removed by the mononuclear phagocyte system; under persistent antigenic stimulation, such as long-term exposure to xenobiotics, impaired clearance may cause CIC accumulation and vascular or tissue deposition, initiating inflammatory cascades and endothelial injury (Stanilova et al., 2001; Nweke et al., 2021). Evidence from occupational cohorts in Ghana and Nigeria indicates that pesticide exposure is associated with altered immunoglobulin profiles and elevated pro-inflammatory mediators (Asare-Donkor et al., 2019; Ibrahim et al., 2023), supporting the use of CICs as indicators of immune activation in exposed individuals.
Nitric oxide (NO), synthesized from L-arginine by nitric-oxide synthase (NOS), regulates vascular tone and immune signaling. During prolonged oxidative challenge, inducible NOS (iNOS) becomes over-expressed, producing excess NO that reacts with superoxide to form peroxynitrite ($ONOO^-$)—a potent oxidant responsible for nitrosative/oxidative stress and cellular damage (Lushchak et al., 2023). Human and animal studies have reported increased NO levels and lipid-peroxidation products following chronic pesticide exposure (Yassin et al., 2022; Ibrahim et al., 2023), reinforcing its utility as a biochemical marker of redox imbalance.

Despite increasing global attention to environmental immunotoxicology, there remains a paucity of data from African farming populations, particularly in Nigeria, where pesticide exposure continues to escalate. This study therefore aimed to compare serum CIC and NO levels between chronically exposed farmers and unexposed controls in Southwestern Nigeria, and to evaluate whether duration of exposure correlates with biomarker levels, thereby testing potential dose–response patterns. The findings are expected to advance biomonitoring frameworks and inform occupational-health policy for agricultural workers within the region.

MATERIALS AND METHODS

Study design

Case control design

Exposure index (EI) was computed as *years of pesticide use × average spraying frequency per year*. Repeat interviews with 20 % of farmers within two weeks yielded a reliability coefficient of 0.89, confirming acceptable recall consistency.

Study Site

Farmers were enrolled randomly from Oyebola Village and Alapako Village village, Obafemi Owode Local government, Ogun state and Akinlaalu village, Ife North Local government, Osun state. Obafemi Owode Local government lies on latitude 6° - 57° North and 3° - 30° East with an area of 1410sqkm. It has a total population estimated to be three hundred and ninety-nine thousand, eight hundred (399,800) (NPC, 2022). Likewise, Ife North Local government lies on latitude Lies on latitude 7° - 31′ North and 4° - 27′ East with an area of 889 sqkm. It has a total population estimated to be one hundred and fifty-three thousand, six hundred and ninety-four (153,694) (NPC, 2022). The inhabitants are predominantly farmers engaging in cash crop agriculture such as cocoa and cashew.

Study Population

The study population comprised adult male farmers residing in Southwestern Nigeria who had been occupationally exposed to pesticides for a minimum of 10 years. These individuals were selected based on their active involvement in farming activities requiring regular pesticide use, including mixing, spraying, and handling of agrochemicals without consistent use of personal protective equipment. A total of 50 pesticide-exposed farmers were recruited as the test group. For comparison, 39 age-matched, non-farming males from the nearby towns with no known occupational or residential exposure to pesticides were selected as the control group. The controls were individuals engaged in professions unrelated to agriculture, such as teaching, civil service, or small-scale trading.

Inclusion Criteria

Exposed

Individuals who met the following inclusion criteria were enrolled into the study:

1. At least 25 years of age.
2. Must be a farmer who have been using pesticides for at least ten years within southwest Nigeria.
3. No history of chronic medical conditions such as hypertension, diabetes mellitus, stroke, chronic kidney disease (CKD), and chronic liver disease (CLD).
4. Provision of written informed consent

Controls

- At least 25 years of age
- Non farmworker and have no history of using pesticides
- No history of chronic medical conditions such as hypertension, diabetes mellitus, stroke, chronic kidney disease (CKD), and chronic liver disease (CLD)
- Provision of written informed consent

Exclusion Criteria for Cases and Controls

Participants who satisfied any of the following criteria were excluded from the study:

- Failure to meet any of the inclusion criteria as appropriate
- Pregnant women
- Refusal to give informed consent

Ethical Consideration

Ethical approval UI/EC/23/0545 (02/09/2024) was obtained from the University of Ibadan/University College Hospital Joint Ethics Committee before the commencement of the study. Also, informed consent was obtained from each study participants before enrolment into the study. All procedures conformed to the Declaration of Helsinki.

Pesticide Exposure Assessment and Data Collection

Structured interviews and questionnaires were used to gather data on types, frequency, and duration of pesticide use. Information on clinical history were also obtained using a structured questionnaire.

Determination of Circulating Immune Complexes (CICs)

Serum levels of circulating immune complexes were determined using the polyethylene glycol (PEG) precipitation method as described by Stanilova *et al*. (2001). Briefly, serum samples were mixed with 3.75% PEG 6000 solution to precipitate immune complexes. The mixture was incubated, centrifuged, and the absorbance of the supernatant was measured at 450 nm using a spectrophotometer. CIC concentrations were expressed in equivalent units per milliliter (Eq/mL) with validated detection limit of 5 µgEq/ml

Results were calibrated using pooled human serum (100 Eq/mL standard); assays were performed in duplicate (CV < 6 %), and quality-control sera were run in each batch.

Determination of Nitric Oxide (NO)

Serum nitric oxide levels were assessed using the Griess reagent assay, based on the method of Green *et al*. (1982). The assay involves the conversion of nitrate to nitrite by nitrate reductase, followed by the formation of a diazonium compound with sulfanilamide and subsequent coupling with N-(1-naphthyl)ethylenediamine to produce a chromophore. The resulting pink color was measured spectrophotometrically at 540 nm. Nitric oxide concentration was expressed in micromoles (µM). The Quality Control range considered was 0.5 µM - 100µM.

Sample Collection

Using aseptical technique, 10ml of venipuncture blood sample was collected into a clean dry plain sample container and kept away from sunlight. The blood sample was allowed to clot, then dislodged and spurned at 3000 rpm for 10 minutes to obtain serum. The serum was collected and dispensed into a dry, chemically clean serum container after which the samples were stored at - 20°c until analyzed.

Statistical Analysis

All data was carefully entered on an excel spreadsheet and assessed for accuracy and completion before statistical analysis. The statistical analysis was done using the Statistical package for social sciences (SPSS) version 27. Before analysis, the data were assessed for Gaussian distribution. Student's t-test was used to determine differences in means of the variables with Gaussian distribution. Mann-Whitney U was used to determine differences in medians of the variables without Gaussian distribution. Correlation between variables was determined using Spearman's Rho correlation. *P*-values less than 0.05 (2-tailed) were considered as statistically significant. Results are presented as median (interquartile range), mean ±standard deviation or number (percentage). P-values less than 0.05 were considered as statistically significant.

RESULTS

The characteristics of the study participants as shown in Table 1. The mean age for farmers is 37.2 ± 8.5 and that of controls 33.6 ± 7.1. The farmers have lower educational attainment, 52% had only primary education, compared to controls, where 92.5% had tertiary education. The farmers include individuals from Nigeria and Benin while controls were all Nigerians.

Most farmers (60%) had 10-19 years of experience with higher proportion using pesticides occasionally, with paraquat, organophosphate and glyphosate being the most common types.54% of farmers did not use personal protective equipment (PPE), and 82% had no pesticide training. 26% of the farmers reported some health issues such as eye irritation and respiratory problems, with limited access to medical care.

Serum levels of circulating immune complexes 86(33.5 – 118.5) (µgEq/ml) in farm workers is significantly higher than 50(32 - 64) (µgEq/ml) in controls (*P*=0.001)

Table 1: Characteristic of Study Participants

Variables	Farm Workers (n=50)	Controls (n=39)
Age (years)		
Mean age±SD	37.62±8.25	32.2±6.8
Educational status (%)		
No formal education	1(2.5%)	0
Primary six	23(57.3%)	0
SSCE	12(30%)	3(10%)
Tertiary	4(10%)	27(90%)
Type of Pesticide Used (%)		
Paraquat	5(12.5%)	N/A
Organophosphate and glyphosate	35(87.5%)	N/A
Pesticide usage training (%)		
Yes	7(17.5%)	N/A
No	33(82.5%)	N/A
Use of PPE (%)		
Yes	19(47.5%)	N/A
No	21(52.5%)	N/A

Values are in number (percentage), Senior Secondary School Certification Examination - SSCE, Personal Protective Equipment - PPE

Serum levels of and nitric oxide levels are significantly higher in farmer workers 78.7(63.01 – 101.92) (μM) relative to controls 48.4(39.99 – 58.63) (μM). p=0.040

As shown in Fig. 1, serum levels of circulating immune complexes and nitric oxide levels are significantly higher in farmers relative to controls.

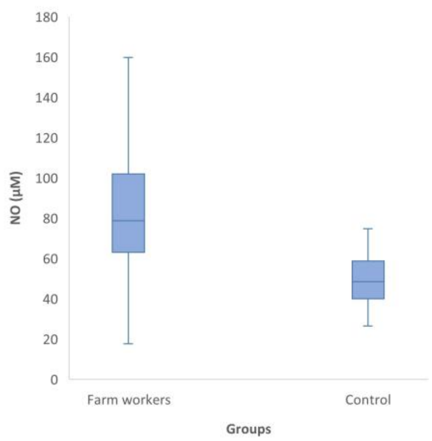

Figure 1a: Serum levels of Nitric oxide levels in farm workers and controls groups((μM)) (P value = 0.000*)

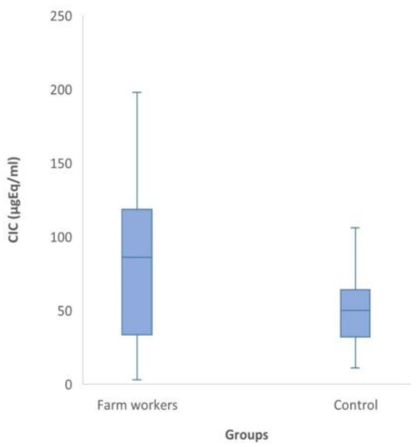

Figure 1b: Serum levels of Circulating immune complexes in farm workers and controls groups(μgEq/ml).

The *P*-values for all correlations are above the standard significance level (0.05), indicating no statistically significant correlation between the duration of pesticide exposure and circulating immune complex, nitric oxide levels parameter in farmers (Table 3 and 4).

Table 2: Correlation between Duration of Pesticide Exposure, Serum Circulating Immune complexes, Nitric oxide levels in farmers and controls

Parameter	Farmers (n =50)	*P*-value
Duration of Pesticide Exposure vs CICs	- 0.050	0.320
Duration of Pesticide Exposurevs NO	0.206	0.151

*Significant at P<0.05, Mann-Whitney U, CICs= Circulating Immune Complexes, NO = Nitric oxide

Table 3: Correlation between nitric oxide and circulating immune complex in farm workers and controls

	r-value	*P*-value
Controls (n=39)	-0.085	0.605
Farm workers (n=50)	0.012	0.934

*Significant at P<0.05, Mann-Whitney U

DISCUSSION

Pesticides remain indispensable in sustaining global food production, yet their chronic use continues to raise major public health concerns, particularly among smallholder farmers in developing countries where enforcement and awareness are limited. This study assessed circulating immune complexes (CICs) and nitric oxide (NO) as biomarkers of immunologic and oxidative stress among pesticide-exposed farmers in Southwestern Nigeria using a case–control design. The significantly higher CIC and NO concentrations observed among exposed participants compared with unexposed controls indicate that prolonged pesticide contact may lead to measurable alterations in immune and redox balance. These findings are consistent with existing literature reporting immune activation and oxidative stress in agricultural workers exposed to mixed pesticides (Ramos et al., 2020; Pathak and Shah, 2022).

The elevated CIC levels observed in this study suggest persistent humoral immune activation and impaired immune-complex clearance, likely resulting from continuous low-dose exposure to agrochemicals. Chronic exposure can induce the formation of pesticide–protein adducts that act as neoantigens, stimulating antibody production and subsequent immune-complex generation. When clearance mechanisms become inefficient, these complexes may deposit in tissues, activating inflammatory pathways and contributing to endothelial or vascular injury (Ohlson et al., 1985). Comparable findings have been reported among pesticide applicators in Asia and Africa, where increased CIC levels and altered immunoglobulin profiles have been linked to prolonged agrochemical exposure (El-Sharkawy and Abdel-Raouf, 2020; Khan et al., 2023). Although complement and cytokine activities were not measured, the elevated CIC values observed serve as a useful composite indicator of immune disturbance associated with pesticide exposure in this population.

Similarly, the elevated NO levels found among exposed farmers reflect enhanced oxidative and nitrosative stress due to chronic pesticide handling. Continuous activation of inducible nitric oxide synthase (iNOS) in response to inflammatory or oxidative stimuli increases NO production, which reacts with superoxide radicals to form peroxynitrite, a potent reactive nitrogen species capable of lipid and protein oxidation. This cascade can impair cellular function and DNA integrity, linking exposure to both immune and oxidative dysfunction (Lushchak et al., 2023). The observed increase in NO therefore provides biochemical evidence of oxidative imbalance, aligning with mechanistic studies in pesticide-exposed workers and laboratory models (Mwangi et al., 2023).

Despite clear group differences in biomarker levels, the study found no significant correlation between the duration of pesticide use and either CIC or NO. This outcome is biologically and methodologically plausible. Duration of use alone may not accurately represent true exposure intensity, as farmers vary in spraying frequency, types of pesticides applied, and adherence to safety precautions. Differences in product formulation, concentration, and environmental persistence may further obscure linear associations. Additionally, biomarkers such as CIC and NO fluctuate in response to recent exposures and physiological status rather than cumulative dose. The case–control design, which compares existing groups rather than following exposure over time, also limits the detection of dose–

response gradients. Comparable findings have been documented in other agricultural settings where exposure heterogeneity weakens duration–effect correlations (Mwangi et al., 2023).

These results carry important implications for Nigeria's agricultural workforce. Many of the farmers in this study lacked formal training in pesticide handling, and fewer than half reported consistent use of personal protective equipment (PPE). Similar trends have been documented across Southwestern Nigeria, where PPE non-use and poor risk awareness remain major contributors to pesticide-related health outcomes (Adegbola et al., 2023; Oshingbade et al., 2025). The combination of limited education, informal pesticide markets, and lack of supervision perpetuates unsafe practices, increasing chronic exposure potential. The biomarker alterations demonstrated here thus offer biological support for existing observational evidence of unsafe pesticide use among Nigerian farmers.

At the policy level, Nigeria has recently taken progressive steps to strengthen pesticide regulation through the National Agency for Food and Drug Administration and Control (NAFDAC). The 2024 Bio-Pesticide Registration and Labelling Regulations emphasize product safety, user education, and environmental protection. Internationally, the Stockholm Convention Conference of the Parties (May 2025) added chlorpyrifos to Annex A for global elimination, signaling growing consensus against highly hazardous pesticides. Together, these developments highlight the urgency of national strategies that combine regulation, farmer training, and exposure monitoring. Integrating simple biomarker screening—such as CIC and NO assays—into occupational health surveillance could serve as an early-warning system for at-risk farmers and guide preventive interventions aligned with the FAO (2024) pesticide-reduction framework.

This study's major strength lies in its application of two complementary, measurable biomarkers reflecting immune and oxidative alterations in a real-world farming population. The case–control design allowed comparison between clearly defined exposure groups, enhancing the validity of observed associations. However, the study also faced limitations, including reliance on self-reported exposure histories and absence of quantitative pesticide residue or metabolite data, which restricts chemical-specific interpretation. Future investigations should adopt longitudinal case–control or cohort designs that incorporate precise exposure metrics such as number of sprays per year, duration per session, and seasonal variation alongside comprehensive biomarker panels to better characterize temporal and mechanistic relationships

Sample size was a limitation in this study. The relatively small number of participants may limit the generalization of the findings. The reliance on self-reported data for pesticide use frequency and duration introduces potential recall bias.

In addition, factors such as nutrition and exposure to other environmental pollutants were not fully controlled for and may have influenced the results. The study did not measure pesticide residues or metabolites in participants, which could have provided more direct evidence of exposure.

CONCLUSION

In conclusion, this study provides evidence that chronic pesticide exposure among Nigerian farmers is associated with elevated serum CIC and NO levels, reflecting immune activation and oxidative stress. The lack of correlation between exposure duration and biomarker concentrations likely reflects heterogeneous exposure patterns rather than absence of effect. These findings underline the need for continuous farmer education, improved PPE availability, stricter enforcement of pesticide regulations, and inclusion of biomarker surveillance in agricultural health programs. Strengthening such initiatives will help safeguard farmworkers' health while promoting sustainable agricultural productivity within Nigeria's evolving regulatory framework.

Additional studies are vital to investigate the prolonged health consequences of pesticide exposure, especially its impact on immune and vascular function. The development of safer, less harmful pesticide substitutes should be prioritized to reduce risks while preserving crop yields. At a wider level, community-based education efforts can inform farmworkers and their households about the dangers of pesticide exposure and the value of safeguards. Partnerships between farming bodies and public health entities are crucial to share expertise, supply resources, and enhance the overall health of farmworkers.

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Data Availability
All data generated are represented in this manuscript.

Conflict of Interest
The authors declare no conflict of interest in this study

Author Contribution
EMO and SAY conceived and designed the study; EMO, OJO, and EOA conducted participant recruitment, sample collection, and laboratory assays; SAY and OJO performed statistical analyses and data interpretation; EMO and EOA drafted the initial manuscript; SAY and OJO critically revised the manuscript for important intellectual content; all authors reviewed and approved the final manuscript. SAY supervised the overall research activity.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process
The authors acknowledge that AI-assisted technologies were partially used in the preparation of this manuscript. Specifically, generative AI tools (ChatGPT and DeepSeek) were employed to support language refinement and grammar correction. All content was critically reviewed, edited, and approved by the authors to ensure accuracy, originality, and alignment with academic standards. No AI tools were used to generate data, interpret results, or replace human intellectual contribution.

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