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Association Between Occupational Pesticide Exposure and Parkinson's Disease Risk: An Observational Study In The South Indian Population

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Abstract

The pathways and molecular mechanisms underlying the impact of prolonged pesticide exposure on Parkinson's disease (PD) pathophysiology remain elusive. Given the association of altered expression levels of transcription factors (TFs) such as NURR1 and FOXA1 with PD, any dysregulation in these TFs could disrupt neuronal maintenance mechanisms, potentially increasing susceptibility to PD. We hypothesize that environmental insults such as long pesticide exposure could interact with the genes encoding for the TFs NURR1 and FOXA1 thereby, perturbing the regulatory mechanisms and maintenance of the midbrain dopaminergic neurons. In our study, we used NURR1 and FOXA1 as our biomarkers for PD. The transcriptomes from the peripheral blood lymphocytes collected from the blood samples of rural agricultural workers who were subjected to long-term pesticide exposure amongst the Indian subpopulation were profiled to observe any aberration in the expression levels of NURR1 and FOXA1. We demonstrated a significant downregulation of Nurr1 & Foxa1 mRNA expression in the pesticide exposure group compared with the healthy controls. This data supports that pesticide exposures may be in the initial stage of dopamine neuron degeneration as it is difficult to measure the motor symptoms of PD using the UPDRS scale in the preliminary stages. However, the current study did not identify the risk factor between male and female groups. In conclusion, our findings provide compelling evidence implicating pesticides in PD risk. This is the first human population study indicating that pesticide exposure may elevate PD risk, utilizing transcription factors as markers. Our population-based retrospective cohort study indicates higher long-term PD risks associated with pesticide exposure, potentially constituting an independent PD risk factor.

Keywords: Parkinson's disease, transcription factors, dopamine neurons, DNA damage, pesticide

Introduction

Parkinson's disease arises from a complex interplay of genetic and environmental factors. Environmental influences, particularly long-term pesticide exposure, have been linked to the sporadic onset of PD in various epidemiological studies (1,2). Notably, the herbicide Paraquat shares molecular similarities with MPP^+ (1-methyl-4-phenylpyridinium) and has been found to specially damage dopaminergic neurons, increase neuronal oxidating stress, impair mitochondrial complex I, and disrupt the ubiquitin-proteasome system (3,4). In addition to paraquat, other pesticides such as rotenone, organochlorines, maneb, and organophosphates have also been associated with an elevated risk of PD (5). Studies indicate that pesticides exposure may not only increase

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the risk of PD but also contribute to earlier onset of symptoms and premature death in PD patients.Glyphosateexposure, for instance, has been linked to motor, cognitive and psychiatric symptoms,suggesting that pesticides may impact all stages of PD progression (6,7). Beyond observational studies, there are reportssuggesting genetic alterations due to pesticide exposure in PD. A meta-analysis has highlighted an increased risk of alterations in various PD pathogenesis-related genes, including GST, PON-1, MDR1, and SNCA genes. However, the precise mechanism remain to be fully elucidated (8).

In previous studies Slotkin *et al.*(9), it was found that pesticide exposure led to alterations in the expression levels of several genetic risk factors associated with PD. Specifically, organophosphate exposure, a prevalent compound in many pesticides,induced transcriptional changes in genes associated linked to PD in both laboratory cell studies and animal models. Additionally, a separate study highlighted oxidative stress and mitochondrial dysfunction among agricultural workers exposed to organophosphates. These findings suggest a potential mechanism by which pesticide exposure may increase susceptibility to developing neurological symptoms, possibly through oxidative stress-induced mitochondrial dysfunction (10). Despite these insights , the precise pathways and molecular mechanisms underlying the impact of long-term pesticide exposure on PD pathophysiology remain incompletely understood.

Parkinson's disease is characterized by degeneration of dopaminergic neurons in the midbrain, leading to various phenotypic manifestations such as reduced locomotor activity, tremors, and postural instability. Transcriptional factors (TFs) play a crucial role in regulating dopamine synthesis, promoting dopaminergic neuron development during embryonic stages, and maintaining their function in adulthood (11). Among these TFs, NURR1 and FOXA1 are particularly for the survival, differentiation, and maintenance of midbrain dopaminergic neurons in the (12-14). NURR1 regulates nuclear-encoded mitochondrial genes, and its dysfunction contributed to mitochondrial dysfunction, a mechanism PD pathophysiology (15). Ablation of NURR1 and FOXA1 results in rapid neuron loss in regions like the substantial nigra pars compacta (SNpc), characteristic of PD. Additionally, these TFs regulate the expression of dopamine-related markers like the DA transporter (DAT), tyrosine hydroxylase (TH), and vesicular monoamine transporter (VMAT2). Studies have shown decreased NURR1 expression levels in peripheral blood lymphocytes of PD patients (16) and genetic variations in NURR1 and FOXA1 are associated with PD risk . Previous research in the in the Indian subpopulation identified the polymorphisms in exon 4 of NURR1 and exon 3 of FOXA1 in male and female PD patients.Furthermore, alterations in TF expression levels were reported among PD patients (17). These findings underscore the potential of NURR1 and FOXA1 as biomarkers for assessing PDrisk.

Given that alterations in the expression levels TFs NURR1 and FOXA1 are linked to PD, it is hypothesised that pesticide exposure may disrupt molecular mechanisms crucial for neuronal maintenance resulting in susceptibility to developing PD. This study specifically hypothesizes that pesticide exposure can potentially disrupt the intricate molecular mechanisms responsible for neuronal maintenance, potentially affecting these TFs. This study aims to assess the risk of PD development among rural agricultural workers in the Indian subpopulation exposed to pesticides. NURR1 and FOXA1 will be utilised as biomarkers to evaluate their expression levels in pesticide- exposed agricultural workers. By investigating this association, the study aims to shed light on the potential link between pesticide exposure, TF alterations, and PD susceptibility in this demographic.

Materials and Methods

Study Design

All human studies were conducted with prior approval from the ethical issue

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committee of the Sathyabama Institute of Science and Technology, Chennai. (Sathyabama University/IHEC/Study no.001). This study utilised a cross-sectional design to examine the effects of prolonged pesticide exposure on the health of agricultural workers in Udumalaipettai, Tirupur district, Tamil Nadu.

Participant Recruitment

Agricultural workers exposed to organochlorines and organophosphates for a minimum of 10 years, aged between35 and 65 years were recruited to participate for the study. Participants were recruited from Udumalaipettai in the Tirupur district of Tamil Nadu.

Inclusion Criteria

The following inclusion criteria were applied:

- Healthy agricultural workers.
- Individuals who had been involved in crop cultivation for at least ten years and had utilised the specified pesticides.
- Age between 35 to 65 years.

Exclusion Criteria

The following exclusion criteria were applied:

- Agricultural workers with pre-existing medical conditions (exception for hypertension and diabetes).
- Individuals above 75 years of age.

The inclusion and exclusion criteria were carefully selected to ensure the enrollment of agricultural workers with significant pesticide exposure while minimizing confounding factors.

Data Collection

Demographic and exposure-related data were collected from the participants through structured questionnaires administered by trained interviewers. Specifically, cases were defined as individuals meeting the following criteria: 1) diagnosed with PD within the past 3 years by a physician; 2) engaged in agriculture and use pesticide and fertilizers; 3) duration of involvement in agriculture? 4) presence of allergies while handling pesticides and fertilizers; 5) absence of other neurological condition or serious psychiatric diseases; 6) not in the final stages of a terminal illness; 7) willingness to participate in the study.

Blood Sample collection

Blood samples were collected under sterile conditions with 1ml drawn using a sterile syringe and tourniquet assistance. The collected blood was immediately transferred to 3ml vials coated with EDTA. To prevent coagulation, the vials were thoroughly shaken and stored at 4˚C. All samples were processed within 24 hours of collection. A total of 40 PBL samples were collected, comprising 17 subjects from the pesticide exposed group with an average age of 51.2 ± 11.19 years and 27 healthy controls (HC) matched by gender, age (55.7 ± 9.2 years), and origin.

1RNA Extraction and cDNA synthesis

Whole blood samples (1 mL) were anticoagulated with ethylene diamine tetra acetic acid (EDTA), and mixed with phosphate-buffered saline (PBS) at a 1:1 ratio. The mixture was then layered over HiSep LSM medium (HiMedia), and centrifuged at 400 ×g for 30 min following the manufacturer's instructions. This process allowed for the isolation of Peripheral blood mononuclear cells (PBMCs), which were subsequently washed in ice-cold PBS for RNA extraction. Total RNA was extracted from PBMCs using the TRIzol Reagent method (Invitrogen) following the manufacturer's protocol. Subsequently, cDNA carried out according to the manufacturer's instructions using Applied Biosystem kit (18).

Expression profiling of Nurr1 and Foxa1

The fluorescent real-time PCR reaction was conducted usingan “Applied Biosystem Step one” instrument and Applied Biosystem power SYBR green with specific primers targeting *Nurr1*and *Foxa1.* The PCR conditions consisted of an initial denaturation step at 95^{o}C for 1 min, followed by 40 cycles

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of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 1 min. β-actin was utilised as an internal control for normalisation of gene expression levels. Real-time PCR data were quantitatively analyzed by using the $2^{-\Delta\Delta Ct}$ method (19).

Statistical analysis

In the statistical analysis, X^2 test was employed to assess the differences in the distributions of gender between the pesticide-exposed group and the control subjects. One- or two-way ANOVA was utilised to evaluate differences in the mean values of relative *Nurr1* and *Foxa1* gene expression levels. Additionally, Unconditional multivariable logistic regression analysis was performed to control for potential confounding such as age and gender.This analysis helps determine whether the observed associations between pesticide exposure and gene expression levels remain significant after adjusting for these confounders.

Estimation of the biochemical parameters

The biochemical parameters in the blood samples of agricultural workers were analyzed using a HematologyAnalyzer (biotech). A 20µl blood sample was utilized for this process.A total of 15 parameters were analysed, including the total white blood cell count (WBC), red blood cell count(RBC), red cell distribution width(RDW), granulocytes levels, monocytes level, platelet count, mean platelet volume(MPV), hemoglobin levels, hematocrit levels (HCT) were employed to analyze the proportion of blood made of cells, procalcitonin levels(PCT) to determine the infection levels, mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH)levels, mean corpuscular hemoglobin concentration(MCHC), platelet distribution width (PDW) for identifying any aberration.

Comet assay

The comet assay was performed on 20 µl blood samples following the protocols outlined by Muniz et al 2007 (20) and Kent et al 1995 (21). DNA damage was assessed by measuring the tail length and tail moment using a UV transilluminator.

Results and Discussion

The study investigated the potential correlation between pesticide exposure and risk PD by examining the expression profiling of Nurr1 and Foxa1, alongside assessing haematological parameters and DNA damage. The participants, predominantly aged over 40 and lacked a family history of PD, were divided into cases and controls. Cases tend to be slightly older than controls, with a higher prevalence of males and fewer years of formal education completed. Furthermore, a large proportion of participants were either non-smokers or former smokers. Throughout agricultural activities, subjects were exposed to various organophosphate pesticides, including dimethoate, oxydemetone ethyl, and quinalphos.

Previous research has suggested a twofold increase in PD risk associated with prolonged exposure to organophosphates compared to other types of pesticide (22, 23). Meta-analysis have consistently demonstrated a significant association between PD and pesticide exposure (24-26). Moreover, certain pesticides have been found to disrupt proteasomal function and promote aggregation of α-synuclein, impacting the dopamine system (3).

Increased rates of gene mutations have been observed in PD patients, with specific genes such as GSTP1, SLC6A3, and MDR1 implicated (27). Animal models exposed to pesticides have exhibited behavioral and motor disturbances resembling with PD (28), along with differential expression of certain genes (29; 17). This study represents the first effort to estimate PD risk and occupational pesticide exposure based on genetic variations using Peripheral blood lymphocytes (PBL).

In our study, we assessed the risk of PD by examining the expression profiling of Nurr1 and Foxa1. Initially, we compared gender-based expression profiles between the pesticide-exposed group and the healthy

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Table 1: Gene expression profiling of *Foxa1* and *Nurr1* gene from peripheral blood of agricultural workers. Data are presented as Mean ± SEM, Gene expression was normalized with the beta-actin gene. (ANOVA, *P<0.05 relative to control)

Gender	TF	R2	CI-95%	p-Value
Male	Nurr1	0.520	0.15-0.54	0.002
	Foxa1	0.740	0.44-0.90	0.001
Female	Nurr1	0.170	0.04-0.62	0.08
	Foxa1	1.170	0.04-0.56	<0.06

control group (Table 1). Our findings revealed a significant downregulation of Nurr1 expression in males by 26.52% ($p<0.05$) and in females by 21.09% in the pesticide-exposed group compared to healthy controls. However, no significant changes were observed in female participants. Similarly, Foxa1 expression was significantly downregulated by 54.40% ($p<0.05$) in the male pesticide-exposed group and by 21.80% ($p>0.05$) in the female pesticide-exposed group compared to healthy male controls.

The summary Odd Ratios (ORs) for developing PD based on the Nurr1 expression profile indicated a decreased risk in males, with a value of 0.52 (95% CI: 0.15-0.54) ($p<0.05$), while in females, the OR was 0.172 (95% CI: 0.04-0.62) ($p>0.05$). Regarding the Foxa1 expression profile, cumulative pesticide exposure in males resulted in an OR of 0.74 (95% CI: 0.44-0.90) ($p<0.05$), whereas in females, the OR was 1.17 (95% CI: -0.04-0.56) ($p>0.05$).

These findings suggest that pesticide exposures may indeed contribute to the initial stages of dopamine neuron degeneration, as evidenced by the observed downregulation of Nurr1 and Foxa1 expression. However, it is challenging to assess motor symptoms of PD solely based on these expression profiles.The Unified Parkinson's Disease Rating Scale (UPDRS) is commonly used to evaluate motor symptoms in PD patients, but its utility in assessing early-stage PD solely based on expression profiles of Nurr1 and Foxa1 may be limited. Other factors and clinical assessments may need to be considered for a comprehensive evaluation of PD symptoms and progression.

An in-vitro study assessing the effect of permethrin pesticide exposure reported an increase in Nurr1 expression, which was counteracted by antioxidants (30). While reports do not suggest differential expression of Foxa1, this study revealed varied expression of Foxa1 in individuals after prolonged exposure, indicating its potential as a biomarker for PD progression during pesticide exposure. Although case-control studies have shown a lower risk factor in female groups, this study did not distinguish between male and female risk factors due to gender-specific differences in dopamine neuron degradation initiation (31, 32). Long-term cognitive disturbances were observed in occupationally exposed individuals even after cessation of work, highlighting the need to consider the long-term neurological effects of pesticide usage in agricultural settings.

Our findings are consistent with previous research indicating that high exposure to chemicals and pesticides can lead to changes in hematological parameters, including alterations in hemoglobin levels and increased red blood cell (RBC) and white blood cell (WBC) counts (33). For example, a study by Garca-Garca et al. (34) observed elevated hematological parameters in intensive agriculture workers exposed to pesticides in South-eastern Spain. Similarly, another study noted increased blood parameters in insecticide factory workers (35). In our study, we observed significant changes in RBC and WBC counts among

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male agricultural workers aged 45 to 65, indicative of potential hematological effects associated with pesticide exposure. However, such effects were not observed in female employees (Table 2). Disorders such as polycythemia, characterized by abnormal rises in RBC levels, and leukemia, characterized by increased WBC levels, are often associated with elevated WBC and RBC counts (36). Despite these observations, our biochemical analysis did not reveal extreme abnormalities beyond the changes in RBC and WBC counts. It's important to note that while hematological measures provide valuable insights, they alone may not conclusively establish toxicological evidence. Nonetheless, they serve as additional parameters in understanding potential variations during disease development and progression.

Our study successfully utilized leukocytes from farmers with occupational pesticide exposure to quantify DNA damage employing the comet assay. DNA damage encompasses alterations in the chemical structure and sequence of DNA, which can occur due to exposure to pesticides, leading to lesions that hinder genome transcription and replication. These lesions can result in mutations or aberrant genomes if left

Table 2: Hematological profile of agricultural workers analyzed from the peripheral blood after exposure to pesticides. Data are presented as Mean ± SEM (ANOVA, *P<0.05 relative to control)

S. No	Grouping	WBC count (×10^3 µl)	RBC count (×10^6 µl)	PLT (×10^3 µL)	MCV (fl)	MCH (pg)	MCHC (g/dl)
	Ref Range	4.5 – 12	4.2 - 6.5	100 -1610	53 – 68	16 – 23	30 – 34
1	s1	9.2 ± 1.05	9.91 ± 0.80	1582± 82.3	55.8±6.2	17.7± 1.2	31.8± 1.42
2	s2	8.2 ± 1.61	11.47 ± 2.12	866± 30.1	60.1± 7.1	17.5± 0.88	30.6± 1.2
3	s3	13.4 ± 2.63	9.05± 0.55	1119± 43.3	55.9± 6.3	19± 2.34	31.4± 1.38
4	s4	13.8 ± 4.13	9.01± 0.43	621± 24.2	59.2± 6.71	18± 1.7	32.1± 1.5
5	s5	16.4 ± 3.21	8.53± 0.2	1231± 40.2	58.01± 6.53	19.7± 2.6	31.1± 1.31
6	s6	21.9 ± 7.72	7.51± 0.2	1166±38.3	59.3± 6.8	20.1± 2.98	33.2± 1.75
7	s7	12.6 ± 2.32	7.85± 0.28	1338± 30.1	61.01± 7.5	18.5± 2.1	33± 1.7
8	s8	15.5 ± 4.12	9.26± 0.7	1086± 33.1	56.4± 6.43	18.5± 2.2	32.9± 1.64
9	s9	12.4 ± 1.88	8.29± 0.5	1203± 39.7	57.03± 6.62	19.9± 2.87	32.6± 1.6
10	s10	7.9 ± 1.10	8.87± 0.45	1205± 40.0	54.9± 5.8	18.1± 1.81	32.7± 1.68
11	s11	3.7 ± 0.18	8.82± 0.8	1361± 48.6	56.5± 6.5	19.9± 2.87	32.1± 1.58
12	s12	15.5 ± 5.2	6.67± 0.41	854± 27.2	68.4± 8.3	17.8± 1.4	29.1± 0.72
13	s13	13.5 ± 3.33	8.52± 1.0	1416± 60.1	56.9± 6.8	17.5± 0.88	31.4± 1.38
14	s14	6.5 ± 1.07	8.32± 1.1	899± 31.2	57.3± 7.0	18.4± 2.0	30.6± 1.2
15	s15	9.6 ± 2.02	8.25± 0.88	915± 39.9	56.9± 6.8	19.2± 2.5	32.4± 1.68
16	s16	9.4 ± 2.0	7.57± 0.4	232± 20.5	60.2± 7.1	17.8± 1.46	32± 1.7
17	s17	10.9 ± 3.1	8.55± 1.31	1052± 40.1	58.2± 6.95	16.4±	30.7± 1.4

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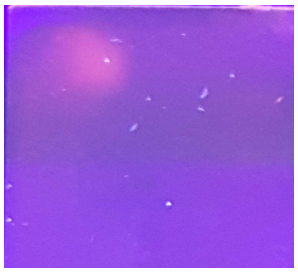

Figure 1: Comet image of blood sample of agricultural workers after exposure to pesticides visualized under UV transilluminator

unrepaired. Upon observation under a UV transilluminator, the presence of comets was evident in the slides [Figure 1], confirming suspected DNA breakage. This highlights the detrimental impact of repeated exposure to pesticides such as organophosphates and organochlorines on DNA structure, leading to DNA damage (37).

Research by Alves et al. demonstrated that workers exposed to complex pesticide mixes exhibited DNA damage as revealed by the comet assay (38). Additionally, soybean field workers exposed to a cocktail of pesticides reported increased DNA damage measured by the comet assay and other biochemical markers (39). Furthermore, malathion, a highly toxic organophosphate pesticide, was shown to cause genotoxic damage to Daphnia magna cells using the comet assay as a biomarker (40, 41). The comet assay serves as a reliable technique for assessing the genotoxicity and carcinogenesis of various agents in human biomonitoring. Notably, our findings suggest that pesticide exposure at work poses a greater risk of DNA damage compared to drinking polluted water, as evidenced by higher levels observed in exposed individuals than in controls (42, 43).

In summary, our study confirms that long-term pesticide exposure has a detrimental effect on DNA structure, leading to DNA breakage, as evidenced by biochemical parameters and the comet assay. However, elucidating the precise impact and pinpointing the effect in Parkinson's disease (PD) studies can be challenging due to the variability in exposure and residual effects of multiple types of pesticides encountered by agricultural workers over time.

Conclusion

In conclusion, our study provides compelling evidence linking pesticide exposure to the risk of Parkinson's disease (PD). By investigating the association between pesticide exposure and PD risk using transcription factors, we have shed light on a novel aspect of this relationship. Specifically, our findings indicate that exposure to pesticides, particularly organophosphates and organochlorines found in pesticides like Dimethoate, Oxydemetone ethyl, and Quinalphos, significantly increases the long-term risk of developing PD.

However, it is crucial to acknowledge the limitations of our study. The sample size may be limited, potentially impacting the generalizability of our results. Moreover, demographic characteristics and occupational biases among participants may introduce confounding variables that influence the observed associations. The reliance on self-reported or indirect measures for pesticide exposure assessment may also introduce recall bias or misclassification.

Despite these limitations, our study underscores the importance of further research in understanding the complex relationship between pesticide exposure and PD risk. Future studies with larger, more diverse samples and rigorous study designs are warranted to validate our findings and elucidate the underlying mechanisms driving this association.

Overall, our study contributes valuable insights into the potential health impacts of pesticide exposure and highlights the need for continued efforts to mitigate pesticide-related risks in occupational settings. By acknowledging these challenges and limitations upfront, we aim to foster

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transparency in the research process and provide a foundation for future investigations in this important area of study.

Authors' Contribution

All authors contributed significantly to the conception and design of the study, as well as the acquisition, analysis, and interpretation of data.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

Data Availability

The data generated and analyzed in this research article are included in the manuscript, and all references in the text are listed in the reference section.

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