ORIGINAL ARTICLE

Exposure to Pesticides and Welding Hastens the Age-at-Onset of Parkinson's Disease

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ABSTRACT: ***Background:*** The age-at-onset (AAO) of Parkinson's disease (PD) is thought to be influenced by environmental factors and polygenic predispositions. Professional exposures to pesticides and toxic metals were shown to be associated with an earlier onset in small sample studies. ***Aim of Study:*** The aim of this study was to confirm the association between professional exposures to pesticides and toxic metals and the AAO of PD, on a larger cohort of patients, defined with a clinic-based ascertainment scheme. ***Methods:*** We used an incident cohort of 290 patients recruited through three designated movement disorder clinics in the province of Quebec, Canada. Patients completed a detailed questionnaire regarding professional exposures to pesticides and toxic metals. We compared the AAO in patients without prior professional exposure ($N = 170$) and those with exposure to pesticides ($N = 53$) or toxic metals through welding ($N = 30$). We further subdivided patients exposed to pesticides according to the frequency and proximity of their contacts. ***Results:*** Patients with prior exposure to pesticides (AAO = 54.74 years) or toxic metals (54.27 years) had a significantly earlier AAO compared to the control group (59.26 years) ($p = 0.003$). In those exposed to pesticides, closer ($p = 0.03$) and more frequent ($p = 0.02$) contacts were negatively correlated with AAO. ***Conclusion:*** Exposure to pesticides and toxic metals were both associated with an earlier onset of PD, an effect that was greater with higher levels of exposure, both in terms of frequency and proximity.

RÉSUMÉ: L'exposition à des pesticides et à des métaux toxiques associés à la soudure diminue l'âge d'apparition de la maladie de Parkinson. ***Contexte:*** Il est courant de penser que l'âge d'apparition de la maladie de Parkinson (MP) est influencé par des facteurs environnementaux et des prédispositions polygéniques. À cet égard, on a montré, dans des études portant sur des échantillons plus restreints, que l'exposition à des pesticides et à des métaux toxiques lors d'une activité professionnelle était associée à un âge d'apparition de cette maladie plus précoce. ***Objectif de l'étude:*** Confirmer cette association à l'aide d'une cohorte de patients plus nombreux, cohorte établie en fonction d'un plan clinique de définition des cas (*clinic-based ascertainment scheme*). ***Méthodes:*** Notre étude a donc reposé sur une cohorte de 290 patients recrutés au Québec au sein de trois cliniques des troubles du mouvement préalablement désignées. Les patients choisis ont alors répondu à un questionnaire complet en ce qui concerne leur exposition à des pesticides et à des métaux toxiques dans le cadre de leur travail. Nous avons ensuite comparé l'âge d'apparition de la MP chez des patients n'ayant pas été exposés à ces éléments (n = 170) à l'âge d'apparition de la MP chez ceux ayant été exposés à des pesticides (n = 53) ou à des métaux toxiques associés à la soudure (n = 30). Plus encore, nous avons subdivisé les patients exposés à des pesticides selon la fréquence et le niveau de proximité de leurs contacts avec ces éléments. ***Résultats:*** L'âge d'apparition de la MP chez les patients préalablement exposés à des pesticides (54,74 ans) ou à des métaux toxiques (54,27 ans) s'est révélé notablement plus précoce en comparaison avec l'âge d'apparition de notre groupe témoin (59,26 ans ; $p = 0{,}003$). Chez ceux ayant été exposés à des pesticides, des contacts plus étroits ($p = 0{,}03$) et plus fréquents ($p = 0{,}02$) ont été corrélés négativement à l'âge d'apparition de la MP. ***Conclusion:*** En somme, l'exposition à des pesticides et à des métaux toxiques a été associée à un âge d'apparition de la MP plus précoce, corrélation qui s'est avérée plus importante avec un accroissement des niveaux d'exposition, qu'il s'agisse de fréquence ou de proximité.

Keywords: Parkinson's disease, Age-at-onset, Environmental factors

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Introduction

Parkinson's disease (PD) is an age-related movement disorder characterized by resting tremor, rigidity, bradykinesia, and postural instability. Its prevalence is dramatically increased after age 60. The etiology of PD is believed to be multifactorial resulting from both genetic and environmental factors, with less than 10% having a causative gene mutation identified.[1,2]

Based on epidemiology and toxicology studies, an important line of research focuses on the role of pesticide exposure associated with the risk of PD.[3–6] Some pesticide families including

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Table 1: Postulated mechanisms of action of toxic substances associated with an increased risk of PD[5,6]

Environmental risk factor	Presumed mechanism/post-mortem and animal studies observations/imaging characteristics
Pesticides	
Paraquat	Tissue damage by setting off a redox cycle that generates toxic superoxide free radicalsPossible synergism with Maneb
Maneb	Unknown mechanism Potential synergism with paraquat
Rotenone	Mitochondrial toxin. Inhibition of complex I of the electron transport chain. Progressive neurodegeneration of dopaminergic and non-dopaminergic neurons and oxidative damage
Organochlorines (dieldrin and β-Hexachlorocyclohexane)	Impaired mitochondrial function and oxidative stress via reactive oxygen species, leading to cell death in the substantia nigra
Organophosphates	Dopaminergic cell loss and microglial activation
Metals	
Iron	Neuromelanin in substantia nigra binds to iron and produces free radicals, and leads to lipid peroxidation and cell death Auto-oxidation of dopamine in substantia nigra neurons, releasing additional free radicals Postmortem PD brains show an increase in iron levels
Manganese	Degeneration of the globus pallidum mediated by disruption of the mitochondria, leading to apoptosis and cell death via formation of highly reactive oxygen species Magnetic resonance imaging revealing T1 hyperintensity in the striatum and globus pallidus and a normal dopamine transporter scan

herbicides such as paraquat, fungicides, insecticides, or rodenticides containing organochlorides and organophosphates can cause serious damage to the nervous system. They contribute to the development of PD by including oxidative stress, mitochondrial dysfunction, α-synuclein fibrillization, and neuronal cell loss.[5,6] In addition, exposure to metals such as iron and manganese (through welding, battery manufacturing, long-term parenteral nutrition, and IV synthetic drug use) has also been associated with an increased risk for PD.[7–10] Postulated mechanisms of action of toxic substances on the pathogenesis of PD are summarized in Table 1 (based on recent reviews from Nandipati and Litvan[5] and Sánchez-Santed et al.[6]).

Other factors reflecting both genetic and environmental influences on PD phenotype are vascular risk factors (VRFs) and sex. Vascular leukoencephalopathy and VRFs, which are thought to be mediated by genetic predispositions and lifestyle variables, predispose to a more severe PD symptomatology and more prominent cognitive features.[11,12] Women were also found to have a delayed onset compared to men (by about 2 years[13,14]), a difference that could be partly explained by differences in intracerebral estrogen levels, leading to higher striatal dopamine levels. This difference in estrogen levels can be explained by an intrinsic lower postsynaptic dopamine D2 receptor affinity in women[15] and by exogenous factors such as past pregnancies, gynecological surgeries, and hormonotherapy.[16]

Although specific genes have been associated with earlier onset forms of PD,[1] the data available regarding the influence of environmental factors on age-at-onset (AAO) are scarce. Earlier, AAO was observed in 15 former welders[17] and in 188 patients with prior exposure to hydrocarbon.[18] Similar results were obtained in 36 patients exposed to pesticides and/or heavy metals.[19] One study focusing on AAO in relation to multiple environmental risk factors was retrieved.[20] The authors studied 203 sibling pairs with PD and found only one environmental factor that influenced AAO, that is, a history of head trauma. However, the authors point out that their results need to be interpreted cautiously since the high genetic contribution to PD in this sample may have overshadowed potential environmental influences.

The aim of the present study is to confirm the hypothesized relation between environmental risk factors (pesticides and toxic metals exposure through welding) and AAO of PD, in a larger cohort, defined with a clinic-based ascertainment scheme.

Methods

Participants

We used an incident cohort of 290 patients recruited through three designated movement disorder clinics in the province of Quebec, Canada. Every index case was evaluated by a neurologist and met the Ward and Gibb[21] criteria for idiopathic PD. Additionally, patients needed to be dopa-responsive and to have been diagnosed no more than 10 years previously. Informed written consent form was obtained for each participant, and the study was approved by the ethics committee of the recruitment centers.

Data Collection

Participants and their partners completed a detailed questionnaire regarding social, professional, and medical history. The questionnaires included sociodemographic data (age, sex, and education), health outcomes at enrolment, and other conditions likely to impact on health like smoking. Patients were asked about their occupational history, especially those at risk for exposures to pesticides. It comprised specific questions regarding any past job in the following fields: manufacturing, farming, forestry, golf or green space maintenance, automobile, chemical products and pesticides spraying, agriculture products, and welding.[20]

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Table 2: Clinical and demographic features of patient's cohort

Characteristics	Control ($N = 170$) (%)	At-risk job ($N = 76$) (%)	Pesticides (direct contact) ($N = 20$) (%)	Welding ($N = 30$) (%)	Total (N = 246) (%)
Gender male N (%)	92 (55)	69 (91)	16 (80)	29 (97)	167 (65)
Age at diagnosis*	59.8 (.98)	54.7 (1.6)	51.2 (2.1)	54.3 (1.8)	58.0 (.69)
History of living near a farm or vegetable garden	115 (68)	55 (72)	14 (70)	19 (63)	170 (69)
Positive history of smoking	83 (48)	32 (42)	12 (60)	14 (46)	120 (49)
High-school or higher education	134 (78)	47 (61)	15 (75)	18 (60)	30 (65)
VRFs					
DM *N* (%)	13 (7.6)	4 (5.3)	0 (0)	2 (6.7)	11 (15 (6.9)
HTN *N* (%)	22 (12)	43 (57)	5 (25)	9 (30)	57 (26)
MI *N* (%)	15 (8.8)	11 (14)	12 (10)	3 (10)	20 (9.2)
DLP *N* (%)	40 (23)	24 (32)	7 (35)	10 (33)	56 (26)
Stroke *N* (%)	2 (1.2)	2 (2.6)	0 (0)	0 (0)	2 (0.9)
>2 RF *N* (%)	29 (17)	18 (23)	4 (20)	8 (27)	175 (81)

DM=diabetes mellitus; HTN=hypertension; MI=myocardial infarction; DLP=dyslipidemia; RF=risk factor.
*Mean (standard deviation).

For each of these past jobs, information on dates of beginning and end of each activity was collected. A positive occupational history was considered when PD patients reported to have experienced at least 6 months of metals and/or pesticides exposition. Patients were asked if they had been exposed to (or directly manipulated) the following: herbicides, fungicides, insecticides, rodenticides, or other unknown substances of similar nature. They rated the frequency and duration of exposures on a 4-point ordinal scale: less than 1/month for <10 years; less than 1/month for >than 10 years; more than 1/month for <10 years; and more than 1/month for >10 years.

Statistical Analysis

The studied cohort consisted of 290 patients from the Quebec City area in eastern Canada. The final analysis included 256 patients whose status regarding professional exposures was detailed. Among these, 76 reported professional exposures to pesticides, toxic metals or both; 20 confirmed directly manipulating pesticides; 30 confirmed being directly exposed to toxic metals through welding; and 4 of them both manipulated pesticides and were former welders.

The dependent variable was age at diagnosis, which is considered to be the most standardized estimate of AAO, free from recall bias and determined by a neurologist. The main independent variable was prior professional exposure to toxic substances. It was further subdivided into nature of the exposure (pesticides and welding), frequency, and proximity. Analysis was conducted with SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

Primary Analysis

The primary analysis focused on two groups of patients: patients without professional exposures (Control, $N = 170$) and those exposed to pesticides or welding (All exposures, $N = 76$). The mean age at diagnosis in both groups was compared with an analysis of covariance (ANCOVA), adjusting for potential confounders that were sufficiently documented in our sample: sex, number of VRFs, place of residence (antecedent of living near a farm or vegetable garden – within 1 km for more than 6 months), which increases the risk of indirect exposure to pesticides, and smoking. Significant covariates would be subsequentially included in secondary group comparisons.

Secondary Analysis

In secondary analyses, the All exposures group was further subdivided into two subgroups (Pesticides, $N = 53$ and Welding, $N = 30$). Both groups were treated independently with separate ANCOVAs, since data regarding pesticides exposure or welding were incomplete in some cases and would have reduced the sample size in each group in a combined analysis; moreover, only four patients had antecedents of both welding and exposure to pesticides, rendering a factorial plan futile.

For the Pesticides group, two "exposure–response" models were tested with multiple linear regression analyses, the first with regards to the proximity of exposures (no contact, $N = 170$; at-risk-job, $N = 33$; and direct manipulation, $N = 20$) and, the second, the frequency of exposures (≤ 1 per month, $N = 12$ and >1 per month, $N = 38$), for a minimal duration of six consecutive months were associated to AAO. The frequency of exposure was chosen over the duration given the potential confounding effect of age on duration, with older patients having longer overall working experience. Significant covariates identified in the ANCOVA would be included in the regression models.

Frequency and proximity analyses were not performed in former welders, since they all had high frequency and proximity exposure to toxic metals.

A α-value of 0.05 was used as the signification threshold for all tests.

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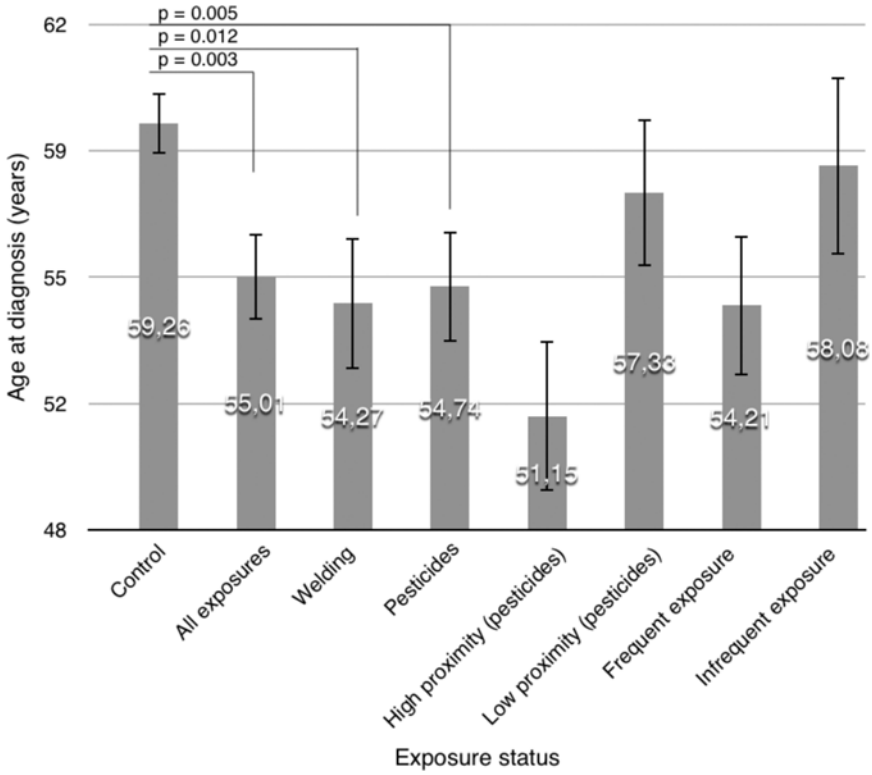

Figure 1: *Age at diagnosis as a function of exposure status. Error bars represent the standard error of the mean. p-values are indicated for all mean comparisons subjected to formal inferential testing.*

Results

Sample Characteristics

The descriptive statistics for the cohort and the subgroups are presented in Table 2. The mean age at diagnosis was 58.0 years (SD = 0.69). The sample included 167 men (65%) and 89 women (35%).

Exposure to Pesticides and Welding Is Associated with Earlier Onset of PD

Homogeneity of the variances among the different cohort subgroups was confirmed with Levene's tests, allowing for the use of parametric tests. Figure 1 presents the mean AAO in the different groups and subgroups. The main ANCOVA included 42 exposed patients and 81 controls in which information about all variables and covariates was sufficient. It revealed a significantly earlier AAO, $F(1, 117) = 9.25$, $p = 0.003$, in exposed patients. The number of VRF was found to be the only significant covariate ($p = 0.0005$; the more VRFs, the latter the AAO).

Subgroup ANCOVAs using the number of VRF as a covariate showed that both pesticides ($N = 65$, $F(1, 231) = 5.76$, $p = 0.017$) and welding ($N = 30$, $F(1, 198) = 17.66$, $p = 0.012$) were associated with earlier onset compared to the control group.

Proximity of Exposures to Pesticides

The mean AAO according to proximity groups is depicted in Figure 1. The linear regression analysis shows that a model including proximity and the number of VRFs significantly predicts AAO ($R^2 = 0.093$, $p = 0.00003$). Proximity is a significant, negatively correlated, independent predictor ($B_s = -0.138$, $p = 0.035$), and the number of VRFs is significantly positively correlated to AAO ($B_s = 0.284$, $p = 0.00002$).

Frequency of Exposures to Pesticides

The mean AAO according to frequency groups is depicted in Figure 1. The linear regression model including frequency and the number of VRFs also significantly predicts AAO ($R^2 = 0.333$, $p = 0.00003$). Proximity is a significant, negatively correlated, independent predictor ($B_s = -0.207$, $p = 0.001$), and the number of VRFs remains significantly positively correlated to AAO ($B_s = 0.266$, $p = 0.00004$).

Discussion

Although there is a clear association in the literature between exposures to pesticides or heavy metals and the risk of PD,[22] how such exposures affect the course of the disease remains unclear. This article reports convincing evidence that occupational exposure to toxic substances influences the AAO of PD, supporting prior evidence of such an association. Exposure to pesticides and welding were both associated with an earlier onset, an effect that was greater with higher levels of exposure, both in terms of frequency and proximity.

Such findings support current – and encourage the development of further – models in which AAO is determined not only by genetic predispositions, but also by exogenous neuronal insults

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over an extended period of time.[2] This represents an important step forward into determining the variables that lead to neuronal death as the brain ages. Environmental exposures have been associated with PD through several mechanisms leading to cellular dysfunction and eventually neuronal death.[5,6] How these insults combine with genetic factors probably represents the key to understanding the AAO of PD.

Epidemiological studies are pivotal to identify the biological targets warranting scientists' attention in order to develop prevention strategies and disease modifying therapies in PD.[23] Our ability to target high-risk individuals is desirable since they are the most likely to benefit from eventual therapeutic options[24] and knowing that some of these are at risk of developing the disease earlier might contribute to optimize the timing of interventions.

Validity of the Study

The main findings of this study are consistent with prior reports in the literature, notably with the results of Ratner et al.[19] who used a data collection method similar to ours. The addition of an "exposure–response" profile and controlling for many variables reported to be associated to PD risk increases the robustness of the observed effects.

One strength of this study resides in the clinic-based ascertainment scheme, since PD requires diagnostic expertize to differentiate it from other types of movement disorders (e.g. essential tremor, Lewy Body disease, and cerebrovascular disease). This approach minimized the risk of contaminating the cohort with misdiagnosed patients.

Using a case-only design also takes away several potential sources of bias, notably recall and sensitivity bias. However, such designs are also prone to other forms of biases. Wilk and Lash[25] outlined that differences in AAO may reflect generational trends in the prevalence of exposure to the risk (or protective) factors. One finding that could be attributable to such a bias in this study is the positive relationship between AAO and VRF. Indeed, since VRFs are associated with a clinically more severe PD syndrome,[11,12] one could have hypothesized that it would lead to an earlier onset, which is contrary to the results obtained in the present study. The simple – and probable – explanation for this seemingly paradoxical result is that older patients from our cohort, as is the case in the general population, have more accumulated VRFs acquired in an age-dependent manner.[26] As regards the effect of occupational exposures on AAO, if such a bias was in play, it would have led to its underestimation. Indeed, exposure to occupational factors should be intrinsically lower in young patients (who have less working experience), which might falsely associate lower exposure levels to earlier AAO. The opposite inclination in our results strengthens their validity. Looking at the frequency of exposures instead of their duration also reduced the potential impact of such a bias.

Limitations

The main limitation of the current study is the relatively small sample size and the missing data in some subgroups, which prevented the use of a full-factorial plan. Multiple regression analyses were used in order to underline an "exposure–response" profile according to the frequency and proximity of exposures. Traditionally, such analyses are performed on much larger sample sizes, although some authors found that a very small numbers of subjects per variable allows adequate estimation of regression coefficients, standard errors, and confidence intervals.[27] The small number of women in the exposure groups did not allow to consider stratified analysis to assess if the observed effects are applicable to both men and women. Although previous findings showed no significant difference in mean age of onset of PD among male and female,[19,20] we could not fully exclude the possibility of a small contribution of sex differences to the main effect.

All potential confounders could not be controlled for since the data were either not included in the questionnaires or incomplete (e.g. physical activity, caffeine, specific medication, ...). The missing data in some subgroups limited our ability to test separately the association between exposure to specific pesticides and the AAO of PD. The lack of genetic data that may influence AAO also limits the appreciation of a potential interaction with the studied environmental factors. Moreover, the questionnaire items regarding frequency and proximity to exposure are rather vague, which possibly leads to heterogeneous exposure levels within the different subgroups.

Conclusion

Although this study reinforces the notion that environmental exposures affect the AAO of PD, epidemiological studies on larger cohorts are still warranted in order to better identify, among people with prior exposures, who is at risk of developing PD, and who will do so earlier. Such studies should notably be aimed at identifying which specific pesticides compounds are particularly noxious and determining if their effect depends on individuals' genetic profiles.

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Disclosures

Dr. ZG-O reports personal fees from Sanofi Genzyme, personal fees from Lysosomal Therapeutics Inc., personal fees from Idorsia, personal fees from Prevail Therapeutics, personal fees from Denali, and personal fees from Inception Sciences, outside the submitted work. The other authors have no conflicts of interest to declare.

Statement of authorship

1. Research project: Conception: P-LG, ND; Organization: P-LG, ND, ZG-O; Execution: P-LG.
2. Statistical analysis: Design and execution: P-LG; Review and critique: ND and ZG-O.
3. Manuscript preparation: First draft: P-LG; review and critique: All authors.

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