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Exposure to pesticides and risk of amyotrophic lateral sclerosis: a population-based case-control study

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Summary. A few epidemiologic studies have suggested an association of agricultural work and pesticides exposure with a severe degenerative disease of the motor neurons, amyotrophic lateral sclerosis (ALS), though conflicting results have also been provided. We investigated through a population-based case-control study the possible relation between overall occupational exposure to pesticides and ALS risk in the northern Italy municipality of Reggio Emilia. By administering a questionnaire, we investigated occupational history and leisure-time habits of the 41 ALS patients diagnosed in the 1995-2006 period, and of 82 age- and sex-matched randomly sampled population controls. More cases than controls were found to have been exposed to pesticides for at least six months (31.7% *vs* 13.4%, respectively), in all cases within the occupational environment. In a conditional logistic regression model, we found an excess ALS risk associated with exposure to pesticides, with a relative risk of 3.6 (95% confidence interval 1.2-10.5). Such association persisted after inclusion in the statistical analysis of potential confounders. Despite the limited statistical stability of the risk estimates, these results appear to indicate that occupational exposure to pesticides is a risk factor for ALS, suggesting the need to further investigate this issue.

Key words: amyotrophic lateral sclerosis, pesticides, epidemiology, case-control studies.

Riassunto *(Esposizione a pesticidi e rischio di sclerosi laterale amiotrofica: uno studio caso-controllo di popolazione)*. Da studi epidemiologici recenti è emersa, sebbene con risultati talvolta contrastanti, una possibile correlazione tra l'esposizione complessiva a pesticidi in ambito occupazionale e il rischio di sclerosi laterale amiotrofica (SLA). Abbiamo studiato questa ipotesi attraverso uno studio caso-controllo nella popolazione del Comune di Reggio Emilia. Abbiamo identificato i 41 nuovi casi di SLA diagnosticati nel periodo 1995-2006 e selezionato nella popolazione generale 82 controlli, appaiati per sesso e età ai pazienti. I soggetti inclusi nell'indagine (o uno stretto familiare quando non altrimenti possibile) hanno compilato un questionario sull'attività professionale e su alcuni fattori dello stile di vita. I pazienti sono risultati caratterizzati da una maggiore esposizione a pesticidi in ambito professionale rispetto ai controlli (rispettivamente 31,7% e 13,4%). Il rischio relativo di SLA associato all'esposizione a pesticidi è risultato pari a 3,6 (intervallo di confidenza al 95% 1,2-10,5), permanendo dopo aggiustamento per alcuni possibili fattori confondenti. Questi risultati suggeriscono nel complesso, nonostante la limitata stabilità statistica delle stime di rischio, una correlazione tra SLA ed esposizione professionale a pesticidi.

Parole chiave: sclerosi laterale amiotrofica, pesticidi, epidemiologia, studi caso-controllo.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare and severe neurodegenerative disease, occurring in sporadic and familial forms, whose etiology is still unknown [1].

Many genetic variants have been investigated as possible risk factors for the familial form, but results are still conflicting [2], while sporadic ALS is considered to be a multifactorial disease with different

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environmental and genetic risk factors suspected to contribute to motor neuron degeneration [3-5].

The association between ALS and exposure to neurotoxic chemicals, such as solvents, some pesticides categories and a few metals and metalloids, has been investigated in several epidemiologic studies with inconsistent results [6-8]; the difficulty in attaining a high level of evidence has been attributed to methodological factors such as inadequate exposure assessment [6]. However, several studies have suggested a relation between exposure to pesticides and ALS risk, and biological plausibility of such association is provided by the well-known neurotoxicity of several of these compounds [8-15].

We investigated through a population-based case-control study the possible relation between occupational exposure to pesticides and ALS risk in an Italian population.

METHODS

We aimed at identifying all residents in the northern Italy municipality of Reggio Emilia (around 150 000 inhabitants) who received a first-time diagnosis of ALS during 1995-2006 period, and provided that they had been resident in the municipality for at least six months, using a methodological approach that has already been described in detail elsewhere [16]. Briefly, we reviewed the hospital discharge register of the Emilia Romagna Region for both inpatients and outpatients of public and private hospitals from 1995 to 2006, as well as the death certificates from 1996 to 2007, and the prescriptions of a drug specific for ALS, riluzole. After having identified the potential cases of the disease, we ascertained the exact diagnosis by reviewing the hospital clinical records when available, or we contacted the general practitioners in case of uncertain diagnosis or missing data. Only the 41 patients fulfilling the El Escorial diagnostic criteria for probable or definite ALS [17], and residing in the Reggio Emilia municipality at the time of diagnosis, were included.

We extracted a group of matched controls from the general population of Reggio Emilia, identifiable through annual directories of residents made available by the General Registry Office of the Region. Using the calendar-year specific file of municipal residents corresponding to the year of diagnosis for each case, we randomly selected two controls matched to the case for year of birth and sex. We administered a questionnaire to cases and controls or, for the deceased cases (39) and controls (13), to the closest available relative, generally the marital/cohabiting partner or a son/daughter. All cases/relatives but one agreed to participate, whilst 11 of the initially sampled 82 controls could not be enrolled, and were replaced by other controls. We collected information about occupational history, and we considered the subject as exposed to pesticides when he/she had been involved in agricultural work and other pesticide-related professional activities for at least six months. We also ascertained occupational exposures to industrial chemicals and magnetic fields, antecedent sources of drinking water, dietary habits, smoking, coffee consumption, physical activity, and history of trauma. Moreover, we collected information about family history of ALS in first-degree relatives, residential history, and educational attainment level, and we checked residential history information reported by the subject against the files of the Municipal Registry Office, also to eventually assess residential exposure to magnetic fields from high-voltage power lines [18].

We estimated the relative risk (RR) of ALS associated with pesticides exposure from odds ratios estimated from conditional logistic regression bivariate and multivariate models, using the statistical package STATA version 10.1 (Stata Corp., TX, 2009).

RESULTS

Forty-one ALS patients and eighty-two age- and sex-matched randomly sampled population controls were eventually enrolled in the study. No occurrence of ALS was reported among the first degree relatives of the cases.

More cases (13/41) than controls (11/82) were found to have been occupationally exposed to pesticides for at least six months in their life (31.7% versus 13.4%, respectively), in all cases due to agricultural work activities. The number of exposed subjects was 10 among cases (33.3%) and 8 among controls (13.3%) in males, while in females 3 cases (27.3%) and 3 controls (13.6%) had been exposed.

In a conditional logistic regression model, we found an excess ALS risk associated with pesticides exposure, with an odds ratio of 3.6 and a 95% confidence interval of 1.2-10.5. Such association persisted after inclusion in the statistical analysis of potential confounders, such as educational attainment level, exposure to chemicals, and residential and occupational exposure to magnetic fields *(Table 1)*.

In sex-specific analysis, the association between pesticide exposure and ALS risk was present both

Table 1 | *Relative risk (RR) with 95% confidence interval (CI) of amyotrophic lateral sclerosis according to occupational exposure to pesticides in conditional logistic regression models, Reggio Emilia municipality, Northern Italy, 1995-2006*

Model	RR	95% CI
Crude	3.6	1.2-10.5
Multivariate – 1[a]	5.3	1.6-17.1
Multivariate – 2[b]	3.3	1.1-9.6
Multivariate – 3[c]	3.7	1.3-11.1
Multivariate – 4[d]	4.7	1.4-15.5

[a] Adjusting for educational attainment level. [b] Adjusting for occupational and residential exposure to magnetic fields. [c] Adjusting for exposure to chemicals. [d] Adjusting for all potential confounders (educational attainment level, exposure to chemicals and magnetic fields).

Table 2 | *Relative risk (RR) with 95% confidence interval (CI) of amyotrophic lateral sclerosis related to occupational pesticides exposure according to sex and age, Reggio Emilia municipality, Northern Italy, 1995-2006*

Model	Sex				Age			
	Males		Females		< 68 yrs		> 68 yrs	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Crude	4.4	1.2-16.7	2.4	0.4-15.0	2.4	0.4-15.0	4.4	1.2-16.7
Multivariate – 1[a]	6.8	1.5-30.1	3.1	0.4-22.4	4.8	0.6-39.1	5.5	1.3-22.9
Multivariate – 2[b]	3.7	1.0-14.4	2.2	0.3-15.4	6.0	0.6-58.2	3.9	1.0-15.1
Multivariate – 3[c]	4.7	1.2-18.8	2.4	0.4-15.0	2.1	0.3-13.0	6.7	1.3-35.8
Multivariate – 4[d]	5.3	1.2-23.9	3.0	0.4-25.7	2.4	0.4-15.5	6.2	1.2-32.7

[a] Adjusting for educational attainment level. [b] Adjusting for occupational and residential exposure to magnetic fields. [c] Adjusting for exposure to chemicals. [d] Adjusting for all potential confounders (educational attainment level, exposure to chemicals and magnetic fields).

in males and in females, though evidence for an excess risk was stronger in males, both in the crude and in the adjusted analysis. After stratifying for age using 68 years (median age at disease onset) as cutoff, the excess ALS risk associated with pesticides exposure was considerably higher in the oldest group *(Table 2)*.

DISCUSSION

In our population-based study, we found a strong association between occupational exposure to pesticides overall considered and ALS risk. In males such association resulted to be stronger than in females, suggesting the influence of sex-related factors, such as metabolic patterns, or different features of the occupational exposures to pesticides occurring in males, though the small number of subjects limits the interpretation of this finding. Moreover, ALS relative risk resulted to be higher in the oldest group, indicating the possibility of an interaction between pesticides and age in favouring ALS onset, possibly due to a long induction period after exposure required to trigger disease onset.

Exposure to pesticides have already been reported to be associated with ALS risk in some investigations, sometimes with evidence of dose-response effects, but inconsistent results have also been reported [8-13, 15]. The hypothesis of an association between exposure to pesticides used in football grounds and ALS risk has also been proposed to explain the increased disease incidence found in soccer players [19-21]. Alternatively, intense physical activity has been suggested as a possible explanation of this observation, but the lack of increased ALS risk in professional road cyclists and basketball players indicates that the disease is not related to physical activity per se [21].

ALS has been previously found to be associated, in few but not all investigations, with other environmental factors such leisure-time habits, education, smoking, electromagnetic fields and chemical agents exposure, physical activities and history of trauma [1]. In our study, inclusion in the statistical analysis of three potential confounders, education and exposure to chemicals and magnetic fields, did not reduce the association between pesticides and ALS risk, but it even enhanced it. When we further adjusted the analysis for additional factors such as consumption of dietary supplements, coffee and alcohol consumption, smoking habits, physical activity, and history of trauma, the result did not change substantially (data not shown).

We found that all ALS cases were of the sporadic form of the disease, which is generally considered to be a multifactorial disease with several genetic and environmental potential risk factors [2-5]. Recent investigations have shown a possible association between sporadic ALS and gene polymorphisms of paraoxonase (PON), an enzyme detoxifying the organophospate pesticides [22, 23]. Exposure to organophosphates, in particular when associated with a genetic tendency to a less efficient detoxification, has been proposed as a possible explanation for ALS increased risk in Gulf War veterans [24]. Nevertheless, in contrast to these studies, a more recent large meta-analysis showed no significant association of ALS risk with the PON locus [25], suggesting the need to further investigate this issue.

Major strengths of our study were the use of multiple sources of data in identifying new cases, including pharmacological prescriptions, the accuracy in confirming diagnosis of ALS and the methodology to identify the controls, matched for age, sex and calendar-year to the cases. On the converse, some limitations of the present investigation must be acknowledged. First, the small size of our sample, as reflected by the low statistical stability of the risk estimates, which is particularly evident in the multivariate analysis. Moreover, we adopted a "crude" measure of exposure to pesticides, *i.e.* a dichotomous indicator of overall occupational history related to use of these compounds, without taking into consideration dose and duration of exposure as well as the specific nerotoxic pesticides to which the subjects may have been exposed [14]. This is a crucial issue, since several but not all the compounds belonging

to the pesticides classes (insecticides, herbicides, fungicides and rodenticides) are recognized neurotoxicants, and even in these cases their neurotoxic effects are due to different and still not entirely understood mechanisms [14, 26]. Therefore, results of the present study should be interpreted as an indication that overall occupational exposure to pesticides is a risk factor for sporadic ALS in this Italian population, but they also suggest the need of further in-depth investigation of this relation in larger studies, focusing on careful assessment of exposure to single pesticides classes or compounds.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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