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Micronucleus analysis in a Portuguese population exposed to pesticides: Preliminary survey

Carla Costa*, Susana Silva, Patrícia Coelho, Joana Roma-Torres, João Paulo Teixeira, Olga Mayan

National Institute of Health, Centre of Environmental and Occupational Health, Praça Coronel Pacheco, 15, 4050-453 Porto, Portugal

Abstract

The general population is exposed in their everyday life to different chemicals namely to pesticides. Many of these compounds are capable of inducing mutations in DNA and lead to several diseases including cancer. With this study we intended to evaluate DNA damage inflicted by pesticide exposure in a population occupationally exposed to those chemicals by means of the micronucleus (MN) test. The obtained results showed a significant increase in MN frequency in occupationally exposed individuals ($p<0.001$) compared with the control group. Higher frequencies of MN were associated with a specific workplace (greenhouses) and the lack of protective measures (gloves) during labour activities. These results reinforce that conditions in workplace should be improved to minimize exposure to these chemicals. This study also emphasizes the need to reinforce the good practices campaigns in order to enlighten those who work with pesticides on the potential hazard of occupational exposure and the importance of using protective measures.

Keywords: Human biomonitoring; Micronucleus; Pesticide exposure

Introduction

The widespread use of pesticides in agriculture represents a threat not only to the environment but also to human populations exposed to them. While some of these compounds target metabolic processes unique to the pests they are intended to fight, most of them are relatively non-specific and poisonous to biological systems common to many species. In most cases, the margin of safety that humans enjoy as users (rather than targets of pesticides) is only a function of body mass (Keifer, 2000).

Although we know that the population with higher levels of exposure to pesticides is the one that directly deals with them in their jobs (pesticide formulation, manufacture and application), everyone is in contact with different amounts of pesticides in normal day life, mainly in edible products. In Portugal these products have a higher use (3.74 kg/ha) than in the average European countries (2.10 kg/ha) (Instituto do Ambiente, 2003).

Different organizations (International Agency for Cancer Research – IARC; and Environmental Protection Agency – EPA) classified several of these chemicals as carcinogenic or mutagenic to humans (IARC, 1991). Cancer is one of the most complex processes in biology. It is a multi-step process that includes initiation, progression and metastasis. None of these states must be seen through a reductionist approach. Carcinogenesis

*Corresponding author. Tel.: +351 222 046 400; fax: +351 222 046 409.
E-mail address: cstcosta@gmail.com (C. Costa).

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is a nonlinear process, whose behaviour does not follow clearly predictable and repeatable pathways (Grizzi and Chiriva-Internati, 2006). However, it is known that the decisive step in carcinogenesis is a result of an irreversible qualitative change in one or more characteristics of cancer cells namely chromosomal changes.

The identification of micronucleus (MN) is an effective tool for genetic damage assessment that has occurred as the result of chromosomal breakage or spindle dysfunction. MN are either acentric chromosome fragments or whole chromosomes that are left behind during mitotic cellular division and appear in the cytoplasm of interphasic cells as small additional nuclei (Surrallés et al., 1995).

Studies performed with the purpose to find genetic damage employing the chromosome aberrations (CA) test, sister chromatid exchange (SCE) analysis and the MN assay in pesticide exposed populations resulted inconclusive. Some of them show an increase in genetic damage (Gómez-Arroyo et al., 2000; Garaj-Vrhovac and Zeljezic, 2002) while others find no differences between these populations and those not exposed to these compounds (Scarpato et al., 1996; Pastor et al., 2001).

The aim of this study was to evaluate potential genotoxic damage in occupational exposure to pesticides by means of MN test. Influences of work behaviours and/or practices on exposure were also studied.

With this study we intended to obtain preliminary results that can enlighten us on the factors that increase exposure. This will allow us to choose the appropriate population to proceed with a larger study with the aim to observe how different forms in genes (polymorphisms) involved in metabolic processes and DNA repair can affect the prevalence of damage in cells (assessed with comet assay, SCE and CA frequency).

Material and methods

Study population and sample collection: The study population consisted of 33 workers exposed to pesticides (17 men and 16 women) in agricultural explorations and greenhouses located in Porto district, Portugal, and 33 non-exposed control employees working mainly in administrative offices in the same area matched by age, sex, lifestyle and smoking habits. The characteristics of both groups are described in Table 1. Each subject gave their informed consent and filled a detailed questionnaire to determine confounding factors as medication, X-rays, etc. Subjects of the exposed group also gave information related to working practices such as use of protective measures, years of employment and possible previous acute intoxications.

Heparinized blood samples (5 ml) were obtained by venipuncture from each donor. All samples were coded and analysed under blind conditions.

Table 1. Characteristics of the study population

	Control group	Exposed group
No. of subjects	33	33
Age (years)[a]	41 ± 9 (22–56)	43 ± 10 (24–77)
Gender	17 male 16 female	17 male 16 female
Years of employment[a]		15 ± 13 (0.5–48)
Smokers	11 (33%)	10 (30%)
Non smokers	22 (67%)	23 (70%)

[a]Mean ± standard deviation.

MN analysis: Aliquots of 0.5 ml heparinized whole blood were cultured and treated as described by Teixeira et al. (2004). To determine the total number of MN in binucleated cells, a total of 1000 binucleated cells with well-preserved cytoplasm (500 per replicate) were scored for each subject. MN were scored blindly by the same reader and identified according to the criteria of Caria et al. (1995) using 500-fold magnification.

Statistical analysis: The distribution of variables was compared with the normal distribution by means of the Kolmogorov–Smirnov goodness-of-fit test. The studied variable departed significantly from normality and therefore the non-parametric Mann–Whitney *U*-test was applied to data. The associations between two variables were analysed by Spearman's correlation. The level of significance was considered 0.05. All analyses were conducted using the SPSS for Windows statistical package, version 11.0.0.

Results

The mean value of MN frequency obtained in the exposed group (9.03 ± 1.04) was significantly higher ($p < 0.001$) compared with the control group (3.27 ± 0.37). The effect of gender was also observed in the control group with females presenting a significant increase in MN frequency ($p < 0.005$) when compared with males. No significant association was found between neither age nor smoking habits with MN frequencies.

When working environment was considered, a statistically significant increase in MN frequency was observed in farmers that worked in greenhouses (14.00 ± 2.80) when compared with those who worked outdoors (7.50 ± 1.08) (Fig. 1). Utilization of individual protective measures during applications influenced the results (although not significantly) being the MN frequency higher in workers that did not use gloves during applications (9.36 ± 1.30) than in those that used this type of protection (8.00 ± 1.35). Duration of exposure had no effect in MN frequencies.

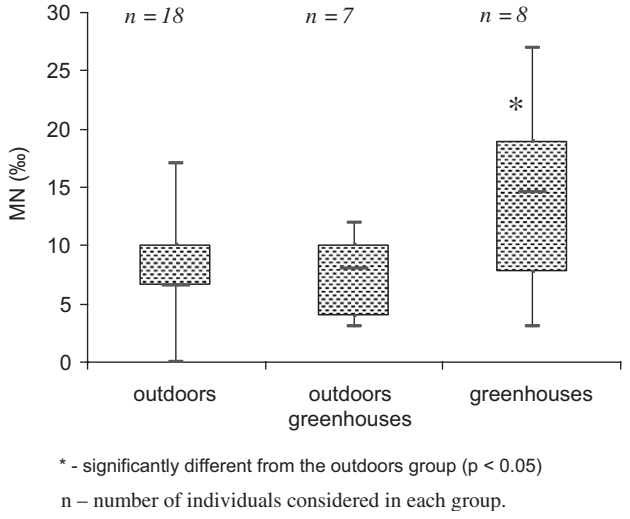

Fig. 1. Boxplot showing frequency distribution of MN (‰) in different working environments within the exposed group.

The vast majority of the subjects included in the exposed group were in contact with a great number of pesticides. For this reason we could not associate the observed damage with a specific product or chemical class.

Discussion

Our results confirm an association between genotoxic damage and occupational exposure to pesticides. Although significance of increased genotoxic effects is difficult to predict for individual subjects the positive findings ensuing from biomonitoring studies suggest a genotoxic hazard at the group level (Bolognesi, 2003).

The gender effect observed within the control group could be explained by supposed preferential aneuploidogenic events involving the X-chromosome (Barale et al., 1998). The X-chromosome is represented in MN more often than expected if equal probability is assumed. Surrallés et al. (1996) reported an excessive overrepresentation of this chromosome in micronuclei of lymphocytes cultured from women. The reason for the decreased inclusion of autosomes and increased inclusion of the X-chromosome in MN of binucleate cells remains unclear.

Smoking habits did not influence MN frequencies in the control and exposed group. Despite the well-known presence of carcinogens in tobacco smoke, results in scientifical literature are controversial. One explanation for the lack of association reported in several studies (Antonucci and Cólus, 2000; D'Arce and Cólus, 2000; Garaj-Vrhovac and Zeljezic, 2002; Márquez et al., 2005) and also in ours is the lower effective concentration of cigarette smoke chemicals in blood than in other organs, such as the lung (Bonassi et al., 2003).

The individuals exposed to pesticides had different working environments: while some worked outdoors, others worked in greenhouses and a group split their time between the two spaces. Results show a significant increase in MN frequency of those who worked in greenhouses when compared to those working outdoors. The occupational pesticide exposure is intense and acute in closed plastic greenhouses without ventilation and therefore considered as high exposures (Gómez-Arroyo et al., 2000). These results are consistent with those described by Bolognesi et al. (2002) where a 28% increase in MN frequency was found in greenhouse workers.

In this study we noticed an increase (although not significant) in MN frequency in individuals that do not use gloves during work activity. This increase may be due not only to the lack of gloves but also the lack of other protective measures and safe practices.

In conclusion, we can say that the results show that pesticides are capable of inducing genetic damage. We could not establish a relationship between damage and specific products but we were able to conclude that the lack of protective measures during working activities as well as the workplace (greenhouses) can be considered risk factors in pesticide exposure.

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