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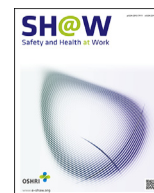

Original Article

Biomonitoring of Toxic Effects of Pesticides in Occupationally Exposed Individuals

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ABSTRACT

Background: Workers in pesticide manufacturing industries are constantly exposed to pesticides. Genetic biomonitoring provides an early identification of potential cancer and genetic diseases in exposed populations. The objectives of this biomonitoring study were to assess DNA damage through comet assay in blood samples collected from industry workers and compare these results with those of classical analytical techniques used for complete blood count analysis.
Methods: Samples from controls ($n = 20$) and exposed workers ($n = 38$) from an industrial area in Multan, Pakistan, were subjected to various tests. Malathion residues in blood samples were measured by gas chromatography.
Results: The exposed workers who were employed in the pesticide manufacturing industry for a longer period (i.e., 13–25 years) had significantly higher DNA tail length (7.04 μm) than the controls (0.94 μm). Workers in the exposed group also had higher white blood cell and red blood cell counts, and lower levels of mean corpuscular hemoglobin (MCH), MCH concentration, and mean corpuscular volume in comparison with normal levels for these parameters. Malathion was not detected in the control group. However, in the exposed group, 72% of whole blood samples had malathion with a mean value of 0.14 mg/L (range 0.01–0.31 mg/L).
Conclusion: We found a strong correlation ($R^2 = 0.91$) between DNA damage in terms of tail length and malathion concentration in blood. Intensive efforts and trainings are thus required to build awareness about safety practices and to change industrial workers' attitude to prevent harmful environmental and anthropogenic effects.
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1. Introduction

Pakistan's agricultural sector holds a significant position in its financial system and accounts for approximately 21% of the country's gross domestic product [1]. A major portion of crop production is lost annually because of the problems associated with growth of weeds, pests, and diseases [2]. This productivity loss coupled with population expansions have led to the increase in use of pesticides. The pesticides used must be toxic to the targeted organisms only. However, various studies have demonstrated the potential hazards of organophosphates, organochlorines, and carbamates for the environment and nontarget species [3]. To reduce their toxic effects, detoxification and biodegradation processes are available, which get activated upon entry of these substances into various compartments of the environment [4,5]. However, inhalation of pesticides remains a serious threat and an important cause of health deterioration in human beings. Exposure to organophosphates could lead to abnormal sperms, fetal death, birth defects, hormonal changes, DNA damage, and changes in ovaries and eggs. Studies in literature have reported that workers exposed to pesticides are more prone to develop leukemia and prostrate, skin, and brain cancers compared with the general population [6,7].

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In general, biomonitoring tools complement the classical methods of monitoring. Genetic biomonitoring provides an early identification of potential cancer and genetic diseases in exposed populations [8]. Many techniques and approaches have been reported for human populations' monitoring [9]. Cytogenetic assays have been used by many researchers to evaluate the potential genotoxic effects of pesticides' exposures in occupationally exposed populations from different countries [10,11]. Interestingly, in populations exposed to pesticides, both positive genotoxic effects [12] and negative findings [13,14] have been reported. The contradictions in the data might depend on the type of pesticide used, protective measures adopted, and/or the cytogenetic end points considered. Comet assay or single-cell gel electrophoresis has been regularly used in biomonitoring studies. It has also been used to test the residual toxicities of biodegradation products [15]. Comet assay is a fast, low-cost, and sensitive tool for assessment of DNA damage (strand break) in single-cell organisms [16]. Generally, human populations have been exposed to different kinds of pesticides almost every day through different routes, including direct or indirect residues in food. However, pesticide workers in manufacturing units and the farmers responsible for spraying pesticides on crops have a high degree of exposure risk and may face health complications. Despite this high risk, only a limited number of studies focusing on the genotoxic effects of exposure to pesticide have been reported [16–18].

Pakistan is an agricultural country, and thus pesticides are produced throughout the year. Consequently, workers in pesticide manufacturing industries are constantly exposed to pesticides. However, only limited work regarding DNA damage at the cell level in exposed workers had been reported in literature [18]. In this context, the purpose of this study was to evaluate DNA damage at the cell level in workers at production units who are constantly exposed to pesticides and highlight the possible risks to the workers. The data generated would help the policy makers to have stringent rules to reduce the exposure risks during occupational activity. The industrial units selected for this study were involved in the production of carbamates, organophosphates, and pyrethroids. The specific objectives of this biomonitoring study were to (1) assess DNA damage through comet assay in blood samples collected from industry workers and (2) compare these results with those of classical analytical techniques used for complete blood count analysis.

2. Materials and methods

2.1. Study groups

A preliminary survey was conducted to identify target groups. The study included 58 individuals. A detailed questionnaire was developed, which was completed through face-to-face interaction. The questionnaire covered very basic questions such as medical history (vaccinations, exposure to X-rays, current treatment if any), demographic data (sex and age), lifestyle habits, working years for exposure period calculations and use of any protective equipment. All the individuals were divided into two groups, namely, control and exposed groups. Twenty healthy individuals (faculty and university students) were grouped into a control group. Through a questionnaire, it was ensured that the selected individuals have not been exposed to any kind of pesticide or other similar agent that can cause serious DNA damage. Thirty-eight individuals from the pesticide industry located in Multan, Pakistan, were included in the exposed group. Based on preliminary data from the questionnaire, smokers were excluded from the sample count. Moreover, individuals with hepatitis C infection were not considered for sampling. The exposure period linked to their employment varied from 6 months to 25 years. During production, all these individuals were simultaneously exposed to a complex mixture of pesticides. All the study participants in the control and exposed groups were informed of the study objectives. The research procedure followed in this study was approved by the Departmental Ethical Committee, Institute of Environmental Sciences and Engineering, National University of Sciences and Technology, Islamabad, Pakistan.

2.2. Blood sample collection

For the study of DNA damage, 5 mL of blood was collected in heparinized tubes containing EDTA as anticoagulant. The samples were collected in the morning at the time when workers logged in at the production plant. This was done to minimize the generation of false-positive results in the comet assay. All the samples were transported in cold chain to the laboratory. Samples were stored at 4°C in clean plastic vials for DNA damage assessments and pesticides analysis. A similar protocol was adapted for the control samples. To avoid interval-based interferences, blood samples from both groups were stored for equal periods, and then processed for further analysis.

2.3. Hematological analysis

Using an automatic analyzer (Medonic, Kobe, Japan), hematological analysis was performed on the freshly collected blood samples. The following hematological parameters were tested in whole blood samples: white blood cells (WBCs), red blood cells (RBCs), and hemoglobin (Hb). In addition, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) were also calculated from the data obtained. MCH gives the average weight of Hb in a single RBC. MCV reflects the size of RBCs by expressing the volume occupied by a single RBC. MCHC measures the average concentration of Hb in RBCs.

2.4. DNA damage analysis using the comet assay

The alkaline comet assay was performed as described by Singh et al [19], but with some minor adjustments. Slides were prepared in duplicate for each sample. Fully frosted microscope slides were covered with 50 μL of 2% normal-melting-point agarose (40–42°C). After application of a coverslip, the slides were allowed to gel at 4°C for 10 minutes. The coverslips were carefully removed, and an aliquot of 10 μL of whole blood and 65 μL of 1% normal-melting-point agarose (37°C) was pipetted onto the precoated slides and allowed to solidify at 4°C for 10 minutes after the application of coverslips. The slides were cleaned and dried, and then a final third low-melting-point agarose layer (75 μL) was applied. Coated slides were kept in a refrigerator at 4°C for 10–15 minutes to let the agarose solidify. Each comet assay run contained randomly selected samples from both controls and workers.

The slides without coverslips were immersed in cold, freshly prepared lysing solution (2.5M NaCl, 100mM EDTA, 10mM Trizma base, 1% Triton X-100, and 10% dimethyl sulfoxide added just before use), and refrigerated at 4°C for 10 hours. The slides were then placed in an alkaline buffer (300mM NaOH and 1mM EDTA, pH $>$ 13) for 20 minutes at room temperature to allow DNA unwinding. Using chilled unwinding TBE buffer (Tris base, boric acid, and EDTA), electrophoresis was conducted for 45 minutes at 25 V (0.74 V/cm), adjusted to 300 mA in a horizontal electrophoresis apparatus (Mupid One, Japan). The slides were then neutralized

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