Mutat Res Fund Mol Mech Mutagen 803–805 (2017) 17–21

Contents lists available at ScienceDirect

Mutat Res Fund Mol Mech Mutagen

journal homepage: www.elsevier.com/locate/mut

DNA damage levels in electronics workers in Southern China: A micro-whole blood comet assay

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ARTICLE INFO

Keywords:
Occupational exposure
Electronics
DNA damage
Micro-whole blood comet assay

ABSTRACT

We evaluated DNA damage levels of different categories of workers exposed to hazards inside electronics factories in Southern China. To find out the most dangerous risk factor, a cross-sectional study was conducted on a total of 584 exposed subjects and 138 controls in an electronics factory in Southern China, where the electronics industry is prevalent. The exposed hazards included isopropanol (IPO), lead, noise, video display terminals (VDT), lead in a high-temperature (high-temp) environment, and IPO in a high-temp environment. DNA damage detection was performed by the micro-whole blood comet assay using peripheral blood. DNA damage levels were estimated by percent tail DNA (%T). Linear regression models were used to test DNA damage differences between exposed groups and control group with adjustments for potential confounding factors. The level of DNA damage was more significant in both lead in a high-temp and IPO in a high-temp environment groups than in that of the controls ($p < 0.05$). The differences remained significant after stratifying by smoking status ($p < 0.05$). There were no significant differences between groups exposed to IPO, lead, noise, VDT environment and controls. In conclusion, we identified potential risk factors for DNA damage to electronics workers. Special attention should be paid to workers exposed to IPO and lead in a high-temp environment.

1. Introduction

The world in which we live runs on electronics; from mobile phones, home appliances and automobiles to worldwide communication and industrial machinery. However, we pay little attention to workers in electronics factories, who suffer from high incidence of occupational diseases due to exposure to various risk factors such as heavy metals, noise, and organic solvents. Occupational diseases related to exposure to risk factors are rapidly spreading in developing countries [1].

With regard to the relevance of individual genetic damage, there is evidence linking the extent of genetic damage to adverse health outcomes [2]. The accumulation of genomic changes by both endogenous and exogenous factors has been recognized as an implied causes of developmental defects and accelerated aging. It has also been linked to increased risk of degenerative conditions such as infertility, immune dysfunction, tumors, and cardiovascular and neurodegenerative diseases [3].

In previous in-vitro studies, we have observed that low levels of lead and arsenic are associated with cellular dysfunction [4,5]. We also found that exposure to low levels of isopropanol (IPO) was correlated with increased arterial blood pressure in a population-based study [6]. Additional research has shown correlations between exposure to low-level risk factors and adverse health outcomes [7–10]. Therefore, there is much to be learned from evaluating whether occupational exposure to exogenous factors in electronics factories plays a role in modulating the levels of genetic damage in factory workers.

In the present study, we extend our previous work using the comet assay to determine the level of genetic damage, since performers can identify DNA breakage at the single-cell level. In the past, surveillance of the population has usually been conducted by isolating lymphocytes from venous blood. Thus, a large blood sample is essential for this procedure, and its procedure is complex [11–14]. In recent years,

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http://dx.doi.org/10.1016/j.mrfmmm.2017.07.005
Received 20 January 2017; Received in revised form 20 June 2017; Accepted 14 July 2017
Available online 22 July 2017
0027-5107/

micro-whole blood comet assay has been applied in risk assessment due to it being simple, fast and sensitive [15–19]. Herein, we performed a micro-whole blood comet assay to estimate the extent of DNA damage in workers exposed to different adverse factors. The overall aim of this study was to explore the extent of DNA damage in groups exposed to different risk factors, as well as to identify the most dangerous occupational exposure factor. Ultimately, we hope to provide clues for genetic risk assessment in the electronics industry.

2. Materials and methods

2.1. Study population

This cross-sectional study was conducted on a total of 722 workers in an electronics factory. 584 were selected as subjects who had been exposed to occupational hazards. 468 subjects (80.1%) were male and 116 (19.9%) were female. Their contact patterns to risk factors included parts assembly, machine operation, welding, polishing, dispensing and cleaning. We divided the subjects into groups according to the different occupational hazards to which they had been exposed, including an IPO group (n = 286), a lead group (n = 165), a noise group (noise was defined as above 85 dB in work environments, n = 38), a VDT group (n = 28), a group which had been exposed to lead in a high-temp environment (high-temp was defined as the work site temperature being 2 °C hotter than the average summer outdoor temperature in a shaded and ventilated area, n = 33) and an IPO in high-temp environment group (n = 34). The control group consisted of 138 subjects including 103 males (74.6%) and 35 females (25.5%)–workers in training who had passed their medical health examinations with no history of occupational exposure to above risk factors. Individuals suffering from high blood lipids, hypertension, acute or chronic infectious and non-communicable diseases were excluded in all groups.

2.2. Occupational health survey and environmental monitoring

The concentration of benzene, acetone, xylene, toluene, and IPO in the air was monitored at multiple work sites using a multi-site sampling method. Lead concentration was sampled using time-weighted average, and the concentrations of benzene, ketone, xylene, toluene, and IPO were sampled using short-term exposure. The monitor data was provided by the Center for Disease Control and Prevention where the factory was located.

2.3. Questionnaire survey and blood sample collection

We designed the “General Health Questionnaire” with on-site labor hygiene survey requirements in mind. Each subject was asked to fill out this questionnaire regarding topics such as age, sex, occupation, smoking habits, alcohol consumption, and duration of exposure.

Technicians collected blood samples (5 mL) were collected in heparinized tubes in strict accordance with the Guidelines. After collection, blood samples were immediately transported to the laboratory for comet testing.

2.4. Comet assay

Comet assay was performed in accordance with the standard protocol previously described [20], but with slight modification. In summary, for each sample, 5 μL of whole blood was mixed with 200 μL of low-melting agarose (1% in phosphate-buffered saline, or PBS). 30 μL of the mixture was put into the well on the comet slides (Trevigen), and then the slides were allowed to gel at 4 °C for 20 min, this was followed by immersion in 4 °C pre-cooled alkaline lysis (2.5 M NaCl,100 mM Na_2EDTA, 10 mM Tris, 10% dimethylsulfoxide (DMSO), 1% triton-X 100, pH ≥ 10) for 3 h. To allow the unwinding of DNA, the slides were placed in alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH = 13) for 20 min. These slides were allowed to undergo further electrophoresis at 25 V (0.8–1.5 V/cm), 300 mA for 30 min at 4 °C in dim light. After electrophoresis, slides were immersed in neutralizing solution (400 mM Tris, pH = 7.5) for 10 min, and this step was repeated. Slides were then dehydrated in absolute methanol for 10 min and kept at room temperature to dry. 60 μL of ethidium bromide (5 μg/mL) was applied over each well of the slides, staining for 25 min in dim light. After staining, slides were immersed in distilled water for 10 min in dim light and this step was repeated. For visualization of DNA damage, slides were observed using a 20× objective fluorescent microscope (NIKON Inverted Fluorescence Microscope equipped with a 460–550 nm excitation filter and a 590 nm barrier filter). A total of 50 individual cells were screened per sample (taken from 10 different fields from each slide) with Comet Assay Software Project (CASP) version 1.2.2. An undamaged cell resembled an intact nucleus without a tail and a damaged cell had the appearance of a comet. The%T was used to evaluate the level of DNA damage, recommended as the best descriptor for DNA break frequencies [20–22].

2.5. Statistical analysis

The data collected were entered into EpiData 3.1 and checked for any inconsistencies, and the results were presented as percentages, mean, and standard deviations. An unpaired *t*-test was used to compare two mean values, and a chi-square test was used to compare dichotomous and categorical variables for demographic characteristics of exposed and unexposed subjects. Linear regression models were used to determine the effect of occupational exposure on DNA damage. $P < 0.05$ was considered statistically significant. All of the analyses were performed using SPSS version 17.0.

3. Results

3.1. Demographic characteristics

A total of 722 blood samples were collected from the IPO group (n = 286), lead exposure group (n = 165), noise group (n = 38), VDT group (n = 28), the group exposed to lead in a high-temp environment (n = 33), the group exposed to IPO in a high-temp environment (n = 34), and the control group (n = 138). The demographic characteristics of both the control and exposure groups are shown in Table 1.

3.2. Reported environmental data suggested low levels of exposure

According to the “GBZ2.1-2007 Occupational exposure limits for hazardous agents in the workplace," reported results showed that lead smoke, acetone, benzene, xylene, toluene, and IPO concentration in the workshops did not exceed the allowable limit (Table 2). This suggests that workers had been exposed to lower concentrations of harmful substances.

3.3. The extent of DNA damage for workers exposed to different hazardous elements

All the parameters of comet assay including tail area, tail DNA, tail DNA%, tail length, comet length, and olive tail moment were analyzed with the CASP comet analysis software, based on the comet images. The linear regression models were used for statistical analysis. The DNA damage levels for the controls and for the different hazard-exposure groups are shown in Table 3. As indicated, DNA damage in subjects observed in the group exposed to lead in a high-temp environment and IPO in a high-temp environment was more significant for the control group.

To evaluate the consistency of these findings by smoking status, we conducted analyses among smokers and non-smokers. Although these