

Genotoxic Monitoring of Nurses Handling Cytotoxic Drugs

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ABSTRACT

Objective: Several biomarkers may be used to detect harmful exposure and individual susceptibility to cancer. Monitoring of biomarkers related to exposure may have a significant effect on early detection of cell transformation, thereby aiding the primary prevention of various chronic and malignant diseases. Nurses who handle cytotoxic drugs are exposed to carcinogenic agents, which have the potential to interrupt the cell cycle and to induce chromosomal aberrations. The presence of high chromosomal aberrations indicates the need for intervention even when exposure to these carcinogens is low. **Methods:** Nationally representative samples of 552 nurses were investigated by a follow-up monitoring system. The measured biomarkers were clinical laboratory routine tests, completed with genotoxicological (chromosome aberrations [CAs] and sister chromatid exchanges [SCEs]) and immunotoxicological monitoring (ratio of lymphocyte subpopulations and lymphocyte activation markers) measured

on peripheral blood lymphocytes. Results were compared to the data of 140 healthy, age-matched controls. **Results:** In nurses exposed to cytostatics, we observed a significantly increased frequency of CAs and SCEs compared with those in the controls. Cytostatic drug exposure also manifested itself in an increased frequency of helper T lymphocytes. Genotoxicological and immunotoxicological changes, as well as negative health effects (i.e., iron deficiency, anemia, and thyroid diseases), increased among cytostatic exposed subjects. **Conclusions:** These results raised concerns about the protection of nursing staff from chemical carcinogens in the working environment.

Key words: Chromosome aberrations, cytostatics, genotoxicological monitoring, health status, immunotoxicology, risk assessment, sister chromatid exchange, unscheduled DNA synthesis

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Introduction

A key aspect of public health is a prompt response to various challenges of environmental attacks, as well as occupational and environmental risk assessment of multiple chemical exposures, infections, and physical and radioactive effects. The effect of environmental chemical and physical agents is significantly evident in a “built” environment. Hazard identification and dose–response relationships have become important in exposure assessment because human data are limited in toxicology. Epidemiology and the use of biomarkers in analytical epidemiology may help bridge the gap between human and animal studies. Quantifying human exposure has also been the starting point for evaluating the usefulness of biomarkers in measuring the effect of environment. Exposure assessment enables the evaluation of genetic polymorphisms responsible for determining why individuals with identical levels of exposure have different degrees of target organ toxicity.

More than four decades ago, concern was expressed regarding the possible hazards to pharmacists and nurses who handled or administered cytostatic drugs.^[1] Most of these drugs have been classified as dangerous to humans because of their mutagenic, clastogenic, and carcinogenic properties.^[2–8]

Primary prevention of occupational diseases is the most powerful tool for health protection in the occupational health sector. Occupational cancer may be preventable by 100% if cancer-causing agents can be eliminated. Detecting early signs of the effects of occupational and environmental carcinogens that cause DNA damage, mutations, and chromosome aberrations (CAs) is a useful indicator of the increased risk of cancer development. The current interpretation of the mechanism of cancer development attributes the appearance of tumors primarily to environmental factors by somatic mutations, and only approximately 5%–10% of the cases are related to original inherent genetic changes. From the aspect of tumor development, the signal transduction mechanism defect is decisive because it is responsible for altering the physiological functions, multiplication, and metabolism of transformed cells. Occupational cancer is induced by exogenous effects, such as chemical, physical, and biological factors, and individual susceptibility promotes the early onset of tumor. The combined effects of these occupational and environmental factors increase the risk of cancer development.

A possible approach for prevention is to eliminate the harmful agents from the (working) environment

(i.e., primary prevention) or to promote the elimination of somatic mutations (i.e., chemoprevention). Chemoprevention can promote apoptosis, increase the activity of DNA repair, or eliminate mutagenic metabolites, for example, with the help of antioxidants.

The objective of our study is to investigate the changes in health status and in the early effects of genotoxic agents of nurses who work in oncology departments and handle cytostatic drugs.

Human genotoxicological monitoring by such methods enables assessment and reduction of genotoxic risks and keeping exposure as low as possible. We have used this complex methodology for the 35 years in the risk assessment of different human populations, control subjects, and persons who are occupationally exposed to various genotoxic agents.^[9–12] This monitoring enables detection and follow-up of alterations in work-related conditions.^[13,14] We have conducted multiple end-point approach genotoxicology monitoring of nurses since 1992 to observe the improvement in their work-related conditions.^[15–17] In this paper, we present the results obtained with the cytogenetic methods, namely, CA and sister chromatid exchange (SCE) and determination of ultraviolet (UV)-light-induced unscheduled DNA-repair synthesis (UDS) levels, together with the results of immunotoxicological methods, in the investigated groups of nurses who prepare and administer cytostatic infusions.

Methods

Donors and sample selection

A total of 552 nurses (1400 tests) who handle cytostatic drugs were investigated and compared with 140 nonexposed age- and gender-matched controls. The controls were all women who work in medical care but were occupationally not exposed to cytostatic drugs.

Each donor was personally interviewed by filling in a routine questionnaire in which they indicated demographic data; smoking and drinking habits; exposure to ionizing radiation and/or to known or suspected chemical mutagens and diseases; and occupational history, including duration of exposure to chemicals and use of protective devices during work. All retrospective medical records were available. Active smoker subjects were considered “smokers.” Written permission was obtained from blood donors, and blood samples were collected from each donor by venipuncture. The samples were processed for cytogenetic analysis, immunophenotyping, and routine clinical check-up.

Cytogenetic analysis

Blood samples were processed for CA and SCE using standard cell culture methods, and 0.8 ml samples of heparinized blood were cultured in duplicates in 10 ml of RPMI-1640 medium (Gibco) supplemented with 20% fetal calf serum (flow) and 0.5% phytohemagglutinin-P (Difco) without antibiotics; then, 5 µg/ml of 5-bromo-2-deoxyuridine (BrdU, Sigma) was added at 22 h of incubation. For CA and SCE analysis, the cultures were incubated at 37°C in the presence of 7% CO₂ for 50 h and 72 h, respectively. Culture harvest, slide preparation, and staining were made following standard methods using 5% Giemsa stain (Fluka) for CA and according to the fluorescence-plus-Giemsa method of Perry and Wolff^[18] for SCE. All microscope analyses were performed on coded slides by the same (two) observers. Characterization of CA was performed according to Carrano and Natarajan^[19] in 100 metaphases per donor in the first mitotic cycle with 46 ± 1 chromosomes. Mitoses that contained only achromatic lesions (gaps) and/or aneuploidy (i.e., 46 ± 1 chromosomes per mitosis) were not considered aberrant. Then, 50 of the second divisions per donor were scored for SCE.

Measurement of ultraviolet-induced DNA repair synthesis

According to the method of Bianchi *et al.*,^[20] UV-induced repair synthesis (UDS) was conducted. The separation of lymphocytes of citrated blood samples was performed by Ficoll–Hypaque density centrifugation. Briefly, PBLs were irradiated in open petri dishes by UV light (24 J/m²) and then incubated for 3 h with 10 µCi/mL 3H-TdR (activity: 37 MBq/mL, Amersham) in the presence of 2.5 mM hydroxyurea. UDS was calculated as the difference between radioactivity in irradiated and control cultures (relative units).

Immunophenotyping

For immunotoxicological investigations, flow cytometry analysis of surface antigens was performed on peripheral blood lymphocytes. Heparinized whole blood was incubated at room temperature for 20 min with appropriate amounts of FITC, PE, PerCP, or APC-labeled monoclonal antibodies (Becton Dickinson) against surface antigens. The erythrocytes were removed by lysis through adding FACS lysing solution (Becton Dickinson). Samples were analyzed within 4 h after labeling or fixed with 2% paraformaldehyde. Four-color analysis was performed on a Becton Dickinson FACSCalibur flow cytometer. Standard forward- and side-scatter gating combined with CD45 was used to separate leukocyte populations and to set the lymphocyte

gate. CD3 was used as T-cell marker, helper T-cells were characterized by CD3+/CD4 + phenotype, cytotoxic T-cells were characterized by CD3+/CD8 + phenotype, and B-lymphocytes were characterized as CD19+ cells. Data for at least 10,000 cells per sample were obtained, and BD CellQuest software version 3.1 © (Becton, Dickinson and Company, Franklin Lakes, New Jersey) was used for analysis.

Statistical analysis

Statistical analyses were performed by the Student's *t*-test with GraphPad Prism 3.02 software (GraphPad Software, Inc. San Diego, California); *P* < 0.05 was considered statistically significant.

Results

Table 1 presents the demography of donors, investigated number of donors, mean age, and percentage of smokers. All donors were of reproductive age between 18 and 50 years.

In the exposed subjects, a decreased mean hemoglobin level only significant at *P* = 0.10 (*P* = 0.0858) and a slightly decreased mean of serum iron concentration, as compared with controls, were observed. Thyroid alterations were not observed among controls, but an elevated incidence of thyroid disease occurred in the exposed group [Table 2]. The mean frequencies of CAs and SCEs were significantly increased in the exposed subjects (*P* < 0.001 and *P* < 0.0005, respectively), as compared with the control group. A slight, insignificant decrease in UDS was also found in the exposed group [Figure 1].

In the exposed group, the percentage of helper T-cells [Table 3] was significantly increased (*P* = 0.001), thereby leading to a significantly elevated Th/Tc ratio (*P* = 0.0183) compared

Table 1: Demography of donors

Exposure	<i>n</i>	Age, years	Smoking, %
Controls	140	38±0.91	25.6
Exposed	552	35±0.44	45.3

Table 2: Mean values of blood hemoglobin and iron, and the number of thyroid disorders among hospital staff exposed to cytostatics

Groups	Mean ± SD		Thyroid disorders
	Hb (mml/L)	Fe (µmol/L)	
Controls (<i>n</i> =140)	8.30±0.08	15.73±0.77	0
Exposed (<i>n</i> =552)	8.15±0.03	14.75±0.32	20

* Statistically significant, Student's *t*-test, *P* < 0.05

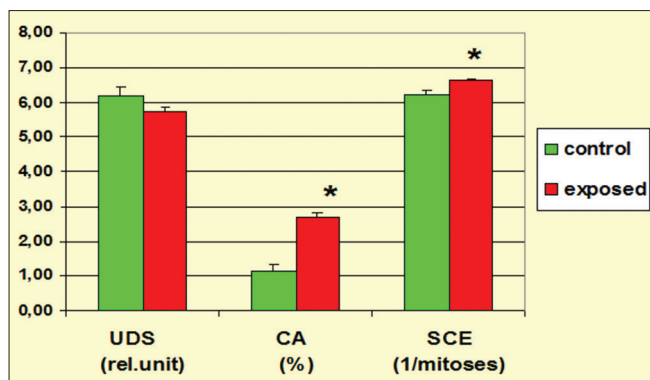


Figure 1: Mean values of genotoxicological investigations among hospital staff exposed to cytostatics. *Statistically significant, Student's t-test, $P < 0.05$

Table 3: Mean values of immunotoxicological investigations among hospital staff exposed to cytostatics

Groups	Mean ± SD				
	T cells (%)	Th cells (%)	Tc cells %	Th/Tc	B cells (%)
Controls	71.59 ± 0.77	42.57 ± 0.76	26.57 ± 0.81	1.77 ± 0.08	10.32 ± 0.44
Exposed	73.02 ± 0.32	45.40 ± 0.37*	25.01 ± 0.31	1.98 ± 0.04*	11.42 ± 0.18*

* Statistically significant, Student's t-test, $P < 0.05$

with the controls. The percentage of B-cells also showed a slight but statistically significant increase ($P = 0.022$).

Conclusion

Hygienic conditions of the working environment are the basic risk factors for the vulnerability of nurses. We observed a significantly increased frequency of CAs and SCEs among nurses exposed to cytostatics. These results agree with the data of oncology nurses and personnel handling cytostatics, with increased frequencies of CAs.^[21-27] An increase in SCEs was also found among nurses who handle cytostatics.^[21,22,28] Cytogenetic and immunological biomarkers are appropriate to detect early susceptibility to diseases. The Hungarian Nurse Study^[6] proved that the use of safety measures could protect against occupational exposure. The increased percentages of CAs, Th cells, and Th/Tc ratio among nurses exposed to cytostatics were even higher among persons with thyroid alterations than those without thyroid alterations, as previously described by us.^[29] Without safety regulations, Hungarian nurses are more susceptible to anemia and thyroid dysfunctions than age-matched controls.

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Conflicts of interest

There are no conflicts of interest.

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