Research Article

Applied Toxicology

Received: 15 June 2011, Revised: 5 August 2011, Accepted: 5 August 2011 Published online in Wiley Online Library: 21 September 2011

(wileyonlinelibrary.com) DOI 10.1002/jat.1735

Evaluation of genotoxicity induced by exposure to antineoplastic drugs in lymphocytes of oncology nurses and pharmacists

Ahmad A. El-Ebiary,[a]* Arwa A. Abuelfadl[a] and Naglaa I. Sarhan[b]

ABSTRACT: The hazards of handling antineoplastic drugs have been raised and discussed in several studies. Introduction of new antineoplastics together with abuse of safety standards have contributed to the exposure risk for personnel who handle these substances. Interactions of antineoplastic drugs with biological structures vary according to the drug(s) and the individual's genetic susceptibility. This study was carried out to evaluate the genome damage induced by exposure to antineoplastic drugs in nurses ($n = 20$) and pharmacists ($n = 18$) working in the Oncology Department of Tanta Cancer Center. Thirty subjects matched in age, gender and smoking habit were selected as controls. Both chromosomal aberration analysis and micronucleus assay were used to evaluate genome damage in peripheral blood lymphocytes of the study subjects. The numbers of aberrant lymphocytes, as well as chromosomal aberration and micronuclei frequencies, were significantly increased in exposed personnel in comparison to matched controls. Compared with pharmacists, nurses showed notably higher level of chromosome damage. On the other hand, no significant difference in micronuclei frequency was observed between nurses and pharmacists. Correlation analyses pointed to the influence of age and duration of occupational exposure on the level of chromosome damage among exposed subjects. The results of this study confirmed that handling antineoplastic drugs without appropriate precautions imposed a genotoxic risk for exposed healthcare workers. These results address the need for regular biomonitoring of exposed personnel. In addition, they call attention to the need for proper implementation of intervention measures aiming to eliminate or significantly reduce worker exposure and prevent untoward biological effects. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: antineoplastic drug; genotoxicity; nurse; pharmacist; lymphocyte; chromosomal aberration; micronucleus assay

INTRODUCTION

Over the last two decades, concern about health hazards of handling antineoplastic drugs has been raised. Some studies have provided evidence of cytotoxicity, genotoxicity and carcinogenicity in exposed subjects (Baker and Connor, 1996; Yoshida *et al*., 2006). Considering the introduction of many new chemotherapeutics and their use as complex mixtures, besides the abuse of safety standards, it would be reasonable to anticipate future higher risks in personnel handling these drugs (Cavallo *et al*., 2005; Kopjar *et al*., 2009).

Unprotected healthcare workers might be exposed to antineoplastic drugs through dermal absorption, ingestion or inhalation resulting from aerosolization of powder or liquid during reconstitution or spillage during preparation or administration to patients. Contact with drug-contaminated equipment or contamination of food or cigarettes from drug on the hands leads to oral ingestion. In addition, patients may excrete these drugs and their metabolites in body wastes, with subsequent exposure of personnel who handle these wastes (Valanis *et al*., 1993).

Since the interactions of antineoplastic drugs with biological structures are manifold and vary according to the drug(s) and genetic susceptibility of the individual, the search for appropriate parameters reflecting biological endpoints of drug effects is an ongoing challenge. Cytogenetic methods provide feasible tools to detect possible effects of long-term exposure at low levels. Among these methods, sister chromatid exchange, chromosomal aberrations (CA) and micronucleus (MN) assays have been frequently applied for quantification of cytogenetic modifications (Pilger *et al*., 2000; Kopjar *et al*., 2009).

According to Rombaldi *et al*. (2009), CA assay, being the most sensitive genotoxicity endpoint, seems to be one of the best techniques to evaluate genetic damage. On the other hand, MN assay is extensively used as a biomarker of chromosomal damage and genome stability in human populations. It enables both chromosome loss and breakage to be measured reliably and is therefore preferred by researchers for assessing chromosomal damage (Rekhadevi *et al*., 2007). Also, it has recently been found to be predictive of cancer risks in human populations (Bonassi *et al*., 2007). Regarding the fact that no single biological marker has been found to be a good indicator of exposure to hazardous drugs or a good predictor of adverse health effects (Baker and Connor, 1996), this study was accomplished using the MN assay to support the evaluation of genome damage carried out by the sensitive CA technique.

Studies on the genotoxicity in healthcare personnel occupationally exposed to antineoplastic drugs have shown contradictory

*Correspondence to: A. A. El-Ebiary, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Tanta University, Tanta, Egypt.
E-mail: a.ebiary@gmail.com

[a]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Tanta University, Tanta, Egypt

[b]Department of Histology, Faculty of Medicine, Tanta University, Tanta, Egypt

Journal of Applied Toxicology

results; both positive and negative findings have been reported (Fucic *et al*., 1998; Maluf and Erdtmann, 2000; Hessel *et al*., 2001; Jakab *et al*., 2001; Burgaz *et al*., 2002; Cavallo *et al*., 2005, 2007; Laffon *et al*., 2005).

Data from one study in one particular occupational setting cannot be used to judge the genotoxicity risk in another occupational setting. This makes this study rational, despite the availability in the literature of investigations of this kind, but on different populations and with different conditions of exposure.

Pharmacists who prepare antineoplastic drugs or nurses who prepare and/or administer them are the two occupational groups who have the highest potential exposure to these agents. Hence, the present study was initiated to assess the genome damage associated with antineoplastic drug handling in oncology nurses and pharmacists working at one of the major cancer centers in Egypt.

MATERIALS AND METHODS

Subjects

Thirty-eight subjects who met the study criteria were selected from health professionals of the oncology department at Tanta Cancer Center. They were grouped as pharmacists ($n = 18$), involved in preparation of antineoplastic drugs at the central pharmacy, and nurses ($n = 20$) assigned to administration of these drugs. This center has a day care unit and oncology wards where the nurses are in continuous rotation (every week) among these sections. As such, participant nurses had similar conditions of exposure, and hence they were dealt with as one group. On average, 550 doses of different drug mixtures are prepared and administered every week. The drugs handled included cyclophosphamide (group 1 of IARC), cisplatin, adriamycin (group 2A), mitomycin C (group 2B), 5-fluorouracil and methotrexate (group 3) (IARC Website, 2011). Thirty volunteer nurses (not handling antineoplastic drugs) of comparable age, gender and smoking habits were selected as controls.

All participants were asked to complete a questionnaire, which included standard demographic data (e.g. age, address, marital state) as well as medical (e.g. health status, exposure to radiation, vaccinations, chemotherapy), lifestyle (e.g. smoking, alcohol, diet) and occupational data (e.g. working hours, duration of exposure). Work characteristics of the exposed subjects, including the use of ventilation hoods and protective equipment (masks, gloves, gowns and goggles), as well as the existence of regulations governing exposure to antineoplastic drugs, were also explored.

All the study subjects were nonsmoking, nonalcohol-consuming females who worked around 7 h per day for 6 days a week. It was ensured that both exposed and control subjects had not received any vaccinations nor had been exposed to any kind of radiation or chemotherapy for 12 months before sample collection. In spite of daily contact, the exact number of hours of antineoplastic drug handling could not be accurately assessed in the exposed subjects.

The study was approved by our local ethics committee. Participation was voluntary, and all participants received detailed information concerning the aims of the research work. Informed consent was obtained from each of them prior to the commencement of the study.

Samples

All subjects contributed to the study with a single blood donation. Venous blood samples (approximately 5 ml) were drawn from each subject into heparinized tubes. Samples were coded and processed within 2 h. Lymphocytes were isolated and washed. Genotoxic effects were evaluated through CA and MN assays.

Methods

Analysis of chromosome aberration

Whole blood culture was performed according to the standard protocol of Watt and Stephen (1986). A 0.5 ml aliquot of heparinized blood was added to the culture medium, which consisted of 5 ml RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 20% heat-inactivated fetal calf serum (Sigma-Aldrich, St Louis, MO, USA) and phytohemagglutinin-M (0.2 ml/5 ml; Gibco, Grand Island, NY, USA). Culture tubes were incubated for 48 h at 37 °C. Colcemid (Sigma-Aldrich, St Louis, MO, USA) was added at a concentration of 0.5 mg ml^{-1} 2 h before harvesting to arrest dividing cells at metaphase. Cells were collected by centrifugation, resuspended in a pre-warmed hypotonic potassium chloride solution (0.075 M) for 10–15 min and fixed in glacial acetic acid–methanol (1:3, v/v). Slides were prepared following air-drying and stained with Giemsa. After staining, the slides were cover-slipped to protect the cells and then stored for scoring. Microscope slides were coded and scored blindly by one observer at 1000× magnification under oil immersion. Two hundred metaphase cells from each individual were analyzed for total numbers and types of aberrations, as well as the percentage of aberrant cells (Buckton and Evans, 1973; Preston *et al*., 1987).

Cytokinesis-blocked micronucleus assay

Cultures were established as described above, where 0.5 ml heparinized blood was added to 5 ml culture medium and incubated at 37°C. The formation of binucleated cells was induced by adding cytochalazine B (Sigma-Aldrich, St Louis, MO, USA) to the cultures after 44 h at a final concentration of 6 μg ml^{-1}. Then the cultures were left in the incubator at 37 °C without interruption for 72 h. At harvest time, cells were centrifuged and the cell pellet resuspended in hypotonic solution of potassium chloride (0.075 M) for 3 min. Afterward, the cells were centrifuged and fixed in a fixative composed of 5 parts of methanol and 1 part of glacial acetic acid. This step was repeated twice. Finally, the centrifuged fixed cells were dropped onto clean, dry slides and stained with Giemsa. After drying, the slides were mounted in DPX and covered with cover slips. Then, slides were coded and scored blindly by one observer at 1000× magnification under oil immersion. One thousand binucleated lymphocytes per individual were scored for the presence of micronuclei (Fenech and Morely, 1985; Surralles and Natarajan, 1997).

Statistical Analysis

The data were analyzed using the SPSS 16.0 program for Windows (SPSS Inc., Chicago, IL, USA).The results were recorded as mean and standard deviation for each group and then statistically analyzed using Student's *t*-test. Correlation analysis was performed using Pearson's correlation test. $P < 0.05$ was considered statistically significant.

Table 1. Characteristics of control and exposed subjects

	Control ($n = 30$)	Exposed		
		Pharmacists ($n = 18$)	Nurses ($n = 20$)	Total of exposed ($n = 38$)
Age (mean ± SD)	30.86 ± 5.77	31.38 ± 4.39	31.1 ± 4.96	31.23 ± 4.64
Years of exposure (mean ± SD)	—	5.8 ± 3.34	10.3 ± 4.52	8.18 ± 4.55

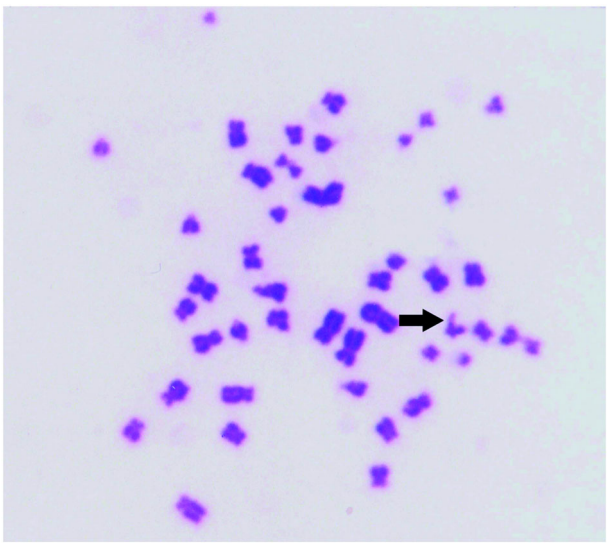

Figure 1. A photomicrograph of a metaphase spread of lymphocytic cultures from exposed group showing gap (Giemsa stain; microscopic magnification, ×1000).

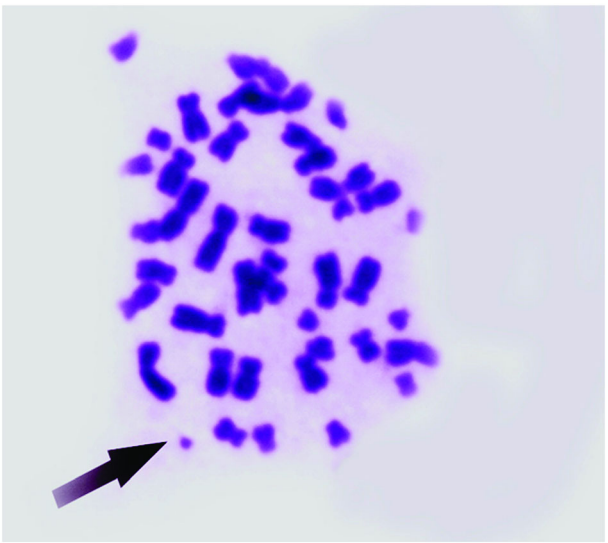

Figure 3. A photomicrograph of a metaphase spread of lymphocytic cultures from exposed group showing fragment (Giemsa stain; microscopic magnification, ×1000).

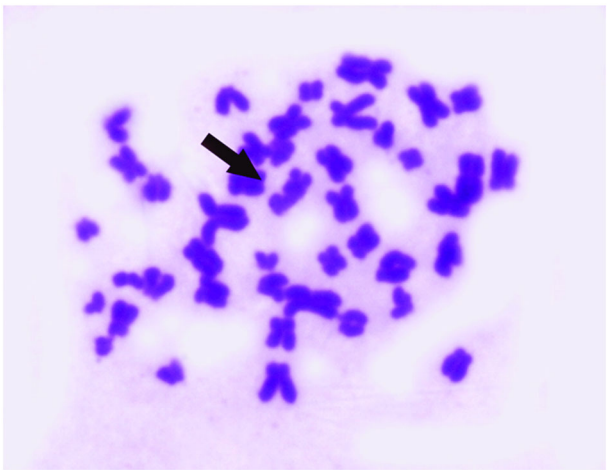

Figure 2. A photomicrograph of a metaphase spread of lymphocytic cultures from exposed group showing break (Giemsa stain; microscopic magnification, ×1000).

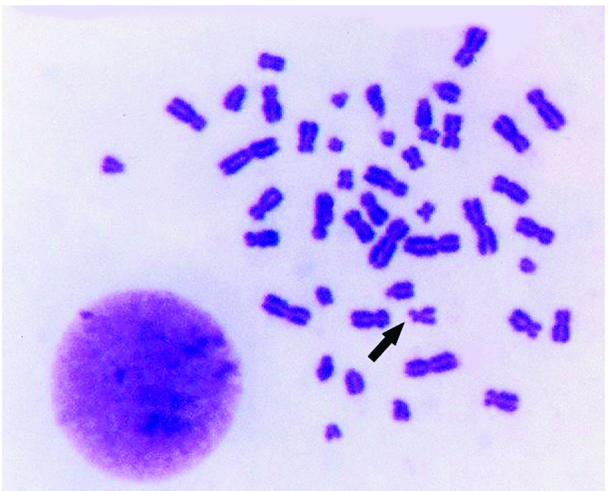

Figure 4. A photomicrograph of a metaphase spread of lymphocytic cultures from exposed group showing deletion (Giemsa stain; microscopic magnification, ×1000).

RESULTS

The main characteristics of the healthcare workers handling antineoplastic drugs and their controls are presented in Table 1. All subjects were age-matched, nonsmoking, nonalcohol-consuming females. According to the information obtained from the questionnaire, the nurses worked in well-ventilated rooms with fans. Gloves were worn during antineoplastic drug administration, but not other safety equipment. Concerning pharmacists, they worked in air-conditioned rooms with safety cabinet facilities for preparing antineoplastic drugs. During drug preparation all of them wore gloves, masks, safety glasses and protective gowns with a closed front and long cuffed sleeves. All exposed subjects had been in contact with antineoplastic drugs for a period of 2–20 years. However, statistical analysis detected a significant difference in years of exposure between nurses and pharmacists ($P = 0.002$).

Journal of Applied Toxicology

Table 2. Mean and standard deviation of total and differential chromosomal aberrations in control and exposed subjects

Subject	Cells scored per subject	Number of aberrant cells	Number of aberrations				Total aberrations per cell	
			Gap	Break	Fragment	Deletion	Excluding gaps	Including gaps
Control	200	1.73 ± 0.22	1.06 ± 0.82	0.43 ± 0.1	0.2 ± 0.08	0.03 ± 0.07	0.69	1.75
Exposed	200	3.36 ± 2.11	1.73 ± 0.24	0.81 ± 0.65	0.5 ± 0.09	0.06 ± 0.04	1.34	3.07
		$P = 0.001$*	$P = 0.03$*	$P = 0.01$*	$P = 0.016$*	$P = 0.004$*	$P = 0.001$*	$P = 0.001$*
Nurses	200	4.7 ± 1.78	2.55 ± 1.57	1.05 ± 0.6	0.65 ± 0.48	0.5 ± 0.11	2.2	4.2
Pharmacists	200	1.88 ± 1.32	0.83 ± 0.61	0.55 ± 0.14	0.33 ± 0.11	0.16 ± 0.09	1.05	1.89
		$P = 0.001$*	$P = 0.001$*	$P = 0.012$*	$P = 0.056$	$P = 0.011$*	$P = 0.001$*	$P = 0.001$*

* Significant $P < 0.05$.

Table 3. Mean and standard deviation of micronuclei in binucleated lymphocytes of control and exposed subjects

Subject	Cells scored/subject	Micronuclei frequency
Control	1000	1.26 ± 0.94
Exposed	1000	2.18 ± 1.29
		$P = 0.001$*
Nurses	1000	$2.3 + 1.41$
Pharmacists	1000	$2.05 + 1.16$
		$P = 0.577$

* Significant $P < 0.05$.

Table 4. Pearson's correlation coefficient for aberrant cells, total chromosomal aberrations and number of micronuclei with age and years of exposure in exposed group

Items		Number of aberrant cells	Total aberrations per cell		Micronuclei frequency
			Excluding gap	Including gap	
Age	*R*	0.054	0.275	0.461	0.074
	P	0.746	0.072	0.004*	0.66
Years of exposure	*R*	0.327	0.043	0.316	0.077
	P	0.045*	0.796	0.053	0.647

* Significant $P < 0.05$.

The numbers of aberrant lymphocytes, as well as CA frequencies, were significantly higher in exposed subjects compared with matched controls. Among the exposed members of this study, a range of aberrations, including gaps (Fig. 1), breaks (Fig. 2), fragments (Fig. 3) and deletions (Fig. 4), was reported. Compared with controls, occupationally exposed subjects had significantly higher levels of all these kinds of aberrations. Likewise, the numbers of aberrant lymphocytes and CA frequencies were considerably higher in exposed nurses compared with pharmacists. All values, except for frequencies of chromosome fragments, were considered statistically significant (Table 2).

Compared with occupationally exposed subjects, controls had notably lower levels of micronucleated lymphocytes. Yet, no significant difference in micronuclei frequency was observed between nurses and pharmacists (Table 3).

Table 5. Pearson's correlation coefficient for aberrant cells and total chromosomal aberrations with number of micronuclei in exposed group

Items		Number of aberrant cells	Total aberrations per cell	
			Excluding gap	Including gap
Micronuclei frequency	R	0.495	0.028	0.102
	P	0.057	0.868	0.544

Correlation analyses pointed to the influence of age on the level of chromosome damage among the exposed subjects, where a significant positive correlation with total chromosomal aberrations including gaps was noticed. In addition, the duration of occupational exposure positively correlated with the number of aberrant cells. However, neither age nor years of occupational exposure significantly influenced the number of binucleated cells with micronuclei (Table 4). In addition, the number of micronuclei showed no significant correlation with the number of aberrant cells or total chromosomal aberrations (Table 5).

DISCUSSION

It is well known that exposure to some of the commonly used antineoplastic drugs is associated with a long list of acute and chronic adverse effects, including cancer (Baker and Connor, 1996; Yoshida *et al*., 2006). Healthcare workers handling these substances usually implement individual and environmental protective measures. However, contamination in the work environment is still possible, and the safety measures employed are not always sufficient to prevent the anticipated health hazards (Turci *et al*., 2003). In addition, not all workers apply the strict measures required for handling such substances. Moreover, based on genetic bases, the human response to genotoxic xenobiotics may vary owing to the presence of individual differences in DNA damage repairing capacity (Berwick and Vineis, 2000). Thus, it is of paramount importance to assess the health hazards to which healthcare workers are exposed owing to the handling of antineoplastic drugs under particular conditions of exposure.

In the present study, a group of health professionals handling antineoplastic drugs was evaluated for genetic damage occurring in peripheral blood lymphocytes through CA and MN assays. Significant increases in chromosomal aberration frequencies,

aberrant cells and cells with micronuclei were detected in exposed personnel compared with controls.

Comparable studies signified the genotoxic potential of antineoplastic drugs through evident genetic damage identified in health professionals handling these drugs. Other studies correlated genotoxic effects and exposure to antineoplastic drugs through environmental as well as biological monitoring (Cavallo *et al*., 2005; Laffon *et al*., 2005; Rekhadevi *et al*., 2007; Rombaldi *et al*., 2009).

However, research on the genotoxicity of antineoplastic drugs displayed conflicting results. Using a variety of assays, several research groups reported either positive or negative findings (Fucic *et al*., 1998; Maluf and Erdtmann, 2000; Hessel *et al*., 2001; Jakab *et al*., 2001; Burgaz *et al*., 2002; Cavallo *et al*., 2005, 2007; Laffon *et al*., 2005; Rekhadevi *et al*., 2007; Rombaldi *et al*., 2009). This inconsistency of the results could be attributed to differences in the antineoplastic drugs handled, the genotoxicity biomarker evaluated or the protective measures employed. It could also be due to the varied conditions to which health professionals were exposed (Rekhadevi *et al*., 2007).

In the current study, although both pharmacists and nurses were regularly handling antineoplastic drugs, pharmacists showed significantly lower frequencies of chromosomal aberrations and aberrant cells. This could be explained by the greater protective measures applied being related to the lower level of genotoxic damage observed (Oestreicher *et al*., 1990; Fuchs *et al*., 1995; Brumen and Horvat, 1996; Kevekordes *et al*., 1998). Antineoplastic drugs are prepared under well-controlled, safer conditions, thus leading to lower exposure levels, which were indeed found at the central pharmacy. At the same time, pharmacists employed more individual protective measures, including the use of gloves, masks, safety glasses and closed gowns with long cuffed sleeves. On the other hand, wearing gloves was the sole protective measure employed by nurses, which was not sufficient to protect against other routes of exposure, especially inhalation of drug aerosols.

Other studies on nurses handling antineoplastic drugs without adequate protective equipment have shown comparable results (Fuchs *et al*., 1995; Brumen and Horvat, 1996; Burgaz *et al*., 2002). In contrast, a few studies (Ensslin *et al*., 1997; Hessel *et al*., 2001) showed no association with genotoxicity in healthcare workers handling antineoplastic drugs. However, this could be attributed to the strictness of the protective measures employed by the study participants.

The difference in the years of exposure between nurses and pharmacists was statistically significant, and this may explain the significantly higher CA frequencies in nurses compared with pharmacists. This is further supported by the presence of significant correlation between years of exposure and aberrant cells, as well as between age and total aberrations, including gaps. These findings could be explained in view of the work of Laffon *et al*. (2005), who proposed a link between long-duration exposure to antineoplastics and the cumulative toxic effect of such agents to the human genome. Likewise, Deng *et al*. (2005) showed comparable increase in genetic damage with the span of continuous work.

The results obtained in this study, as well as studies by other authors, point to possible association between work-related exposure to antineoplastic drugs and genotoxicity. Serious health risk may arise, seeing that data are accumulating to support the concept that genotoxicity endpoints are predictors of human cancer risk. There was experimental and epidemiological evidence for the association of increased frequency of CA in peripheral blood lymphocytes with an increased overall risk for cancer (Hagmar *et al*., 1998). Also, it was reported that increased MN frequency in peripheral blood lymphocytes was a predictive biomarker of cancer risk (Bonassi *et al*., 2007).

The present handling practices of antineoplastic drugs applied in Tanta Cancer Center may not be sufficient to avoid exposure. Data reported herein call attention to the importance of employing adequate safety measures, the proper use of safety equipment and training of personnel prior to employment in order to avoid or lessen potential health hazards caused by antineoplastic drug handling. Moreover, our findings address the need for regular biomonitoring of personnel chronically exposed to antineoplastic drugs, thus contributing to an enhanced health risk assessment and management.

Further research to measure the urinary concentration of antineoplastic drugs and/or their metabolites is needed in order to accurately evaluate healthcare workers' exposure to these drugs. Moreover, investigations of the level of environmental contamination in different healthcare settings are recommended as this may permit correlation of the genotoxic effects with external exposure.

Acknowledgments

The authors thank Dr Ibrahim A. Seifeldin (former director of Tanta Cancer Centre) for his cooperation and assistance throughout the study and also all volunteers who participated in this study.

References

Baker ES, Connor TH. 1996. Monitoring occupational exposure to cancer chemotherapy drugs. *Am. J. Health Syst. Pharm.* **53**: 2713–2723.

Berwick M, Vineis P. 2000. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl. Cancer Inst.* **92**: 874–897.

Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Cebulska-Wasilewska A, Fabianova E, Fucic A, Hagmar L, Joksic G, Martelli A, Migliore L, Mirkova E, Scarfi MR, Zijno A, Norppa H, Fenech M. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* **28**: 625–631.

Brumen V, Horvat D. 1996. Work environment influence on cytostatics induced genotoxicity in oncologic nurses. *Am. J. Ind. Med.* **30**: 67–71.

Buckton KE, Evans HJ. 1973. *Methods for the Analysis of Human Chromosome Aberrations*. World Health Organization: Geneva.

Burgaz S, Karahalil B, Canli Z, Terzioglu F, Ancel G, Anzion RBM, Bos RP, Huttner E. 2002. Assessment of genotoxic damage in nurses occupationally exposed to antineoplastics by the analysis of chromosomal aberrations. *Hum. Exp. Toxicol.* **21**: 129–135.

Cavallo D, Ursini CL, Perniconi B, Francesco AD, Giglio M, Rubino FM, Marinaccio A, Iavicoli S. 2005. Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees. *Mutat. Res.* **587**: 45–51.

Cavallo D, Ursini CL, Omodeo-Sale E, Iavicoli S. 2007. Micronucleus induction and FISH analysis in buccal cells and lymphocytes of nurses administering antineoplastic drugs. *Mutat. Res.* **628**: 11–18.

Deng H, Zhang M, He J, Wu W, Jin L, Zheng W, Lou J, Wang B. 2005. Investigating genetic damage in workers occupationally exposed to methotrexate using three genetic end-points. *Mutagenesis* **20**: 351–357.

Ensslin AS, Huber R, Pethran A, Rommelt H, Schierl R, Kulka U, Fruhmann G. 1997. Biological monitoring of hospital pharmacy personnel occupationally exposed to cytostatic drugs: urinary excretion and cytogenetics study. *Int. Arch. Occup. Environ. Health* **70**: 205–208.

Fenech M, Morely AA. 1985. Measurement of micronuclei in lymphocytes. *Mutat. Res.* **147**: 29–36.

Fuchs J, Hengstler JG, Jung D, Hiltl G, Konietzko J, Oesch F. 1995. DNA damage in nurses handling antineoplastic agents. *Mutat. Res.* **342**: 17–23.

Fucic A, Jazbec A, Mijic A, Seso-Simic D, Tomek R. 1998. Cytogenetic consequences after occupational exposure to antineoplastic drugs. *Mutat. Res.* **416**: 59–66.

Hagmar L, Bonassi S, Stromberg U, Brogger A, Knudsen LE, Norppa H, Reuterwall C. 1998. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res.* **58**: 4117–4121.

Hessel H, Radon K, Pethran A, Maisch B, Grombmair S, Sautter I, Fruhmann G. 2001. The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs – evaluation by the micronucleus assay. *Mutat. Res.* **497**: 101–109.

IARC. Available from: http://monographs.iarc.fr/index.php (accessed 29 May 2011).

Jakab MG, Major J, Tompa A. 2001. Follow-up genotoxicological monitoring of nurses handling antineoplastic drugs. *J. Toxicol. Environ. Health* **62**: 307–318.

Kevekordes S, Gebel TW, Hellwig M, Dames W, Dunkelberg H. 1998. Human effect monitoring in cases of occupational exposure to antineoplastic drugs: a method comparison. *Occup. Environ. Med.* **55**: 145–149.

Kopjar N, Garaj-Vrhovac V, Kasuba V, Rozgaj R, Ramic S, Pavlica V, Zeljezic D. 2009. Assessment of genotoxic risks in Croatian health care workers occupationally exposed to cytotoxic drugs: a multi-bio marker approach. *Int. J. Hyg. Environ. Health* **212**: 414–431.

Laffon B, Teixeira JP, Silva S, Loureiro J, Torres J, Pa´saro E, Me´ndez J, Mayan O. 2005. Genotoxic effects in a population of nurses handling antineoplastic drugs, and relationship with genetic polymorphisms in DNA repair enzymes. *Am. J. Ind. Med.* **48**: 128–136.

Maluf SW, Erdtmann B. 2000. Follow up study of the genetic damage in lymphocytes of pharmacists and nurses handling antineoplastic drugs evaluated by cytokinesis-block micronuclei analysis and single cell gel electrophoresis assay. *Mutat. Res.* **471**: 21–27.

Oestreicher U, Stephan G, Glatzel M. 1990. Chromosome and SCE analysis in peripheral lymphocytes of persons occupationally exposed to cytostatic drugs handled with and without use of safety covers. *Mutat. Res.* **242**: 271–277.

Pilger A, Kohler I, Stettner H, Mader RM, Rizovski B, Terkola R, Diem E, Franz-Hainzl E, Konnaris C, Valic E, Rudiger HW. 2000. Long-term monitoring of sister chromatid exchanges and micronucleus frequencies in pharmacy personnel occupationally exposed to cytostatic drugs. *Int. Arch. Occup. Environ. Health* **73**: 442–448.

Preston RJ, Dean BS, Galloway S, Holden H, McFee AF, Shelby M. 1987. Mammalian in vivo cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mutat. Res.* **189**: 157–165.

Rekhadevi PV, Sailaja N, Chandrasekhar M, Mahboob M, Rahman MF, Grover P. 2007. Genotoxicity assessment in oncology nurses handling anti-neoplastic drugs. *Mutagenesis* **22**: 395–401.

Rombaldi F, Cassini C, Salvador M, Saffi J, Erdtmann B. 2009. Occupational risk assessment of genotoxicity and oxidative stress in workers handling anti-neoplastic drugs during a working week. *Mutagenesis* **24**: 143–148.

Surralles J, Natarajan AT. 1997. Human lymphocytes micronucleus assay in Europe. An international survey. *Mutat. Res.* **392**: 165–174.

Turci R, Sottani C, Spagnoli G, Minoia C. 2003. Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. *J. Chromatogr.* **789**: 169–209.

Valanis B, Vollmer WM, Labuhn KT, Glass AG. 1993. Acute symptoms associated with antineoplastic drug handling among nurses. *Cancer Nurs.* **16**: 288–295.

Watt JL, Stephen GS. 1986. Lymphocyte culture for chromosome analysis. In *Human Cytogenetics: a Practical Approach*, Rooney DE, Czepulkowski BH (eds). IRL Press: Oxford; 39–49.

Yoshida J, Kosaka H, Tomioka K, Kumagai S. 2006. Genotoxic risks to nurses from contamination of the work environment with antineoplastic drugs in Japan. *J. Occup. Health* **48**: 517–522.