

Assessment of DNA Damage in Japanese Nurses Handling Antineoplastic Drugs by the Comet Assay

Makiko SASAKI^{1,2}, Miwako DAKEISHI¹, Shigeko HOSHI³, Noriko ISHII² and Katsuyuki MURATA¹

¹Department of Environmental Health Sciences, Akita University School of Medicine, ²Department of Basic Nursing, Akita University School of Health Sciences and ³Miyagi Cancer Center, Japan

Abstract: Assessment of DNA Damage in Japanese Nurses Handling Antineoplastic Drugs by the Comet Assay: Makiko SASAKI, *et al.* Department of Environmental Health Sciences, Akita University School of Medicine—To clarify genotoxic effects of occupational exposure to antineoplastic drugs in Japan, we examined DNA damage, assessed by the comet assay, in 121 female nurses and 46 female clerks working at three hospitals in the northeast of Japan. The comet assay is considered to be a sensitive and rapid method for DNA strand break detection in individual cells, and tail length and tail moment are used as the comet parameters. Concerning the basal characteristics, the 46 control subjects had higher rates of smoking and coffee-drinking habits and lower hemoglobin than the 121 nurses ($p < 0.05$). The log-transformed tail length in the nurses was significantly longer than that in the control subjects after adjusting for possible covariates such as age and smoking habit ($p < 0.05$). Also, the log-transformed tail length was significantly longer, in the 57 nurses who had handled antineoplastic drugs in the last six months, than that in the 46 control subjects ($p < 0.05$); but, no significant difference in tail length or tail moment was seen between the two nurse groups with and without experience of handling hazardous drugs ($p > 0.05$). These results suggest that Japanese nurses who have worked at hospitals using antineoplastic drugs may have a potential risk of DNA damage. To minimize this risk in Japan, use of biological safety cabinet and appropriate protective equipments, in addition to staff education and training, should be implemented in the healthcare environment.

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Key words: DNA damage, Comet assay, Nurse, Antineoplastic drug

Antineoplastic drugs have paradoxical effects on cancer patients and workers. Specifically, occupational exposure to hazardous drugs can be expected for workers occupied with the production of these drugs but also for nurses preparing and administering them to individual cancer patients. For this reason, many healthcare workers in Europe and America have concerns about the risk to their health through occupational exposure to such drugs. After Falck *et al.*¹ first published data showing enhanced urine mutagenicity of nurses working with cytostatic drugs, various adverse effects such as identification of antineoplastic drugs (*e.g.*, cyclophosphamide) in urine^{2–6}, increased mutagenic activity by the AMES test⁷, chromosomal aberrations^{8, 9}, abnormality of the micronucleus test with lymphocytes and exfoliated buccal cells^{5, 9, 10}, and high frequency of sister chromatid exchanges¹¹, have been demonstrated in healthcare workers exposed to these drugs. Most of these drugs have been proven to have a genotoxic, mutagenic or carcinogenic activity in experimental systems¹², and the US Department of Health and Human Services has also identified some hazardous drugs, such as cyclophosphamide, chlorambucil, melphalan, and tamoxifen, as human carcinogens¹³. The Occupational Safety and Health Administration (OSHA) has presented work practice guidelines for personnel dealing with such drugs¹⁴.

The handling of antineoplastic drugs and its safety awareness may differ among countries and individual hospitals. Apart from the foreign studies noted above, recent research in Japan has reported that about 40% of Japanese nurses are not at all aware of the potential adverse effects of occupational exposure to antineoplastic drugs, though the nurses were selected by nursing department administrators from among nurses who

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Correspondence to: K. Murata, Department of Environmental Health Sciences, Akita University School of Medicine, 1–1–1 Hondo, Akita 010-8543, Japan (e-mail: winestem@med.akita-u.ac.jp)

worked at 13 different cancer centers, 107 university hospitals, and 193 general hospitals with over 300 beds and at least five or more clinics in Japan¹⁵). This figure of 40% may therefore be a conservative estimate due to sampling bias, suggesting that half or more of Japanese nurses do not recognize antineoplastic drugs as occupational hazards. For comprehending the actual state in Japan, it is crucial to clarify whether handling these drugs has damaged individual cells of Japanese healthcare workers, especially nurses. In fact, such research has rarely been performed in Japan^{16, 17}).

As a technique for assessing human DNA damage due to antineoplastic drugs, the comet assay was developed in an empirical way, with two basically different protocols. One method was developed to measure low levels of strand breaks with high sensitivity¹⁸), and another method was optimized to detect a subpopulation of cells with varying sensitivity to drugs or radiation¹⁹⁻²¹). Comets are formed upon the principle of releasing damaged DNA from the core of the nucleus during electrophoresis. In the simple form of Singh *et al.*, the comet assay requires few steps: First, cells are embedded in agarose on a microscope slide. In the agarose, the cells and nuclear membranes are lysed and the DNA is subjected to alkaline electrophoresis. Cellular DNA is visualized using a fluorescent microscope after staining cells with an appropriate dye²¹). In this study, we examined DNA damage, assessed by the comet assay, in Japanese nurses to investigate the genotoxic effect of occupational exposure to antineoplastic drugs quantitatively.

Subjects and Methods

Study population

The study population consisted of 121 female nurses and 46 female clerks (control subjects) who worked at three general hospitals using antineoplastic drugs in northeastern Japan, and who participated voluntarily. General exclusion criteria, such as disorders affecting DNA damage of peripheral blood lymphocytes, were applied. For this reason, we excluded seven nurses who had been exposed to radiation at levels of more than 0.1 mSv/month by dosimeter, and five subjects who did not reply to all questions of the self-rating questionnaire. Of the 121 nurses, 57 had been engaged in preparing or handling antineoplastic drugs for six months or more, and the remaining nurses had not been engaged in such work for at least six months. The work places of the control subjects were far from the clinical departments of the nurses in each hospital, and the control subjects were unlikely to have had any contact with antineoplastic drugs. The research protocol was approved by the ethical review committee of the Akita University School of Medicine, and the nature of the procedure used in the present study was fully explained to all subjects, and the study was carried out with their written informed consent.

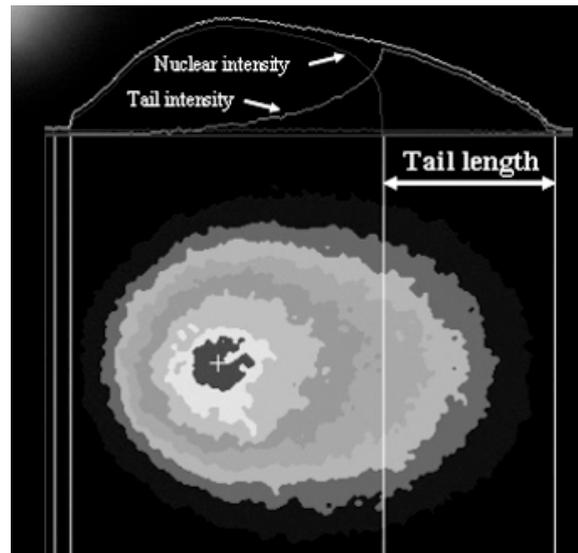


Fig. 1. Evaluation of DNA damage using Comet Analyzer (Youworks, Japan). Tail length was calculated automatically from nuclear intensity and tail intensity of individual cells.

Methods

A detailed survey was conducted using a self-rating questionnaire. The items were medical records (*i.e.*, recent viral diseases, vaccinations, irradiation, past and present history of illness, and medication), work conditions (*e.g.*, kinds of antineoplastic drugs, preparation and administration of antineoplastic drugs, the frequency of handling, kinds of safety precautions applied) and lifestyles (*i.e.*, marital status, smoking, drinking and exercise habits, and supplement intake). To measure the comet assay and liver enzyme function, cubital venous blood samples with sodium heparin were obtained from all subjects in the morning of the day or day-off shift. As an additional prerequisite, the subject must have either been off or worked only a day shift the previous day. At the same time, blood samples with EDTA were collected to count blood cells. All blood samples were coded before blood analysis and these measurements were made by one trained examiner.

Within 24 h after blood collection, peripheral lymphocytes were separated from whole blood utilizing Lymphocyte Separation Media (MP Biomedicals, USA), washed in phosphate buffered saline (PBS) three times and suspended in PBS. The comet assay was carried out under alkaline conditions, basically as described by CometAssayTM protocol of Trevigen instructions²²). Electrophoresis was conducted for 60 min at 300 mA. One hundred cells per subject were examined at a 400 × magnification under a fluorescent microscope (ECLIPSE50i, Nikon, Japan), and the analysis was

Table 1. Basal characteristics of 121 Japanese nurses and 46 control subjects (Mean \pm SD)

	Nurses	Control subjects	<i>p</i> value*
Age (yr)	37 \pm 10	36 \pm 9	0.271
Married (%)	52.1	47.8	0.622
Smokers (%)	9.9	26.1	0.008
Coffee drinkers (%)	71.9	87.0	0.042
Alcohol drinkers (%)	66.9	69.6	0.909
Liver enzyme activities (IU/l)			
Aspartate aminotransferase	22.4 \pm 8.2	19.8 \pm 2.9	0.044
Alanine aminotransferase	18.6 \pm 15.6	15.9 \pm 5.6	0.288
γ -glutamyltranspeptidase	22.8 \pm 23.8	18.4 \pm 7.7	0.274
White blood cells (μ l)	5558 \pm 1728	5594 \pm 1251	0.957
Red blood cells ($10^4/\mu$ l)	449 \pm 36	450 \pm 40	0.928
Platelets ($10^4/\mu$ l)	28.9 \pm 21.6	28.6 \pm 8.8	0.946
Hemoglobin (g/dl)	12.1 \pm 0.9	11.6 \pm 1.5	0.007
Hematocrit (%)	39.2 \pm 2.7	38.2 \pm 4.1	0.081

* Student's *t* test or the χ^2 test was used.

performed automatically using Comet Analyzer Ver. 1.5 (Youworks, Japan). Two global comet parameters were employed to quantify DNA damage; that is, tail length (Fig. 1) and tail moment. The tail length was calculated from the tailing edge of the nucleus to the leading edge of the tail. The tail moment was calculated as tail distance \times ratio (*i.e.*, sum of tail intensity/sum of cell intensity).

Liver enzyme activities of aspartate and alanine aminotransferases (AST and ALT) and γ -glutamyltranspeptidase (γ -GTP) were measured by the JSCC (Japan Society of Clinical Chemistry) standard method with use of an automated clinical chemistry analyzer Fuji Dry-Chem 3500 (Fujifilm Medical Co., Japan). White blood cells, red blood cells, hemoglobin (Hb), hematocrit, and platelets were measured using an automatic blood cell counter MEK-6308 (Nihon Kohden Co., Japan).

Statistical analysis

The comparison of basal characteristics between the nurses and control subjects was made by Student's *t* test or the χ^2 test. Also, the significance of differences in tail length and tail moment between the nurses and control subjects (and between the nurses handling and not handling antineoplastic drugs) was calculated by the analysis of covariance to adjust for possible covariates such as age, smoking and coffee-drinking habits, Hb, experience of irradiation, and hospital. Logarithmic transformations of tail length and tail moment were used because the outcome parameters were highly skewed to the right. Smoking (or coffee-drinking) habit was scored as "nonsmoker or ex-smoker (or nondrinker)"=0 and "smoker (or coffee drinker)"=1; also, irradiation was

scored as "no experience of irradiation, such as X-ray test, within one month"=0 and "experience of irradiation within one month"=1. Since the frequencies of handling antineoplastic drugs and using protective equipments seemed to differ among the three hospitals, these hospitals were assigned to two dummy variables; that is, (0, 0) for the Hospital A, (1, 0) for the Hospital B, and (0, 1) for the Hospital C. All analyses, with two-sided *p* values, were performed using the Statistical Package for the Biosciences²³.

Results

Table 1 presents the basal characteristics of the 121 nurses and 46 control subjects. There were no significant differences in all characteristics except for proportions of smokers and coffee-drinkers, AST activity, and Hb between the nurses and control subjects. Namely, the control subjects had higher rates of smoking and coffee-drinking habits and lower Hb than the nurses ($p < 0.05$). Of these basal characteristics showing significant differences between the two groups, the high AST activity in the nurses seemed to be an outcome due to alcohol-drinking habit or occupational exposure to antineoplastic drugs, rather than a covariate, and it was excluded from possible covariates.

The log-transformed tail length in the 121 nurses was significantly longer than that in the 46 control subjects after adjusting for possible covariates (Table 2). Likewise, the log-transformed tail length was significantly longer in the 57 nurses who had handled antineoplastic drugs for at least six months than in the 46 control subjects (Fig. 2). On the other hand, the log-transformed tail length and tail moment (mean \pm standard deviation (SD)) in the

Table 2. Tail length and tail moment of comets in peripheral lymphocytes in 121 Japanese nurses and 46 control subjects (Mean \pm SD)*

	Nurses	Control subjects	<i>p</i> value
log[tail length (μ m)]	0.764 \pm 0.121	0.711 \pm 0.089	0.020
log[tail moment]	0.312 \pm 0.253	0.253 \pm 0.237	0.239

* Analysis of covariance was used to adjust for age, smoking and coffee-drinking habits, hemoglobin, experience of irradiation, and hospital.

64 nurses not handling those drugs were 0.75 ± 0.10 and 0.28 ± 0.23 , respectively, and no significant differences in the comet assay parameters were seen between the two nurse subgroups (analysis of covariance, $p > 0.05$). Also, the differences in them between the nurse subgroup not handling antineoplastic drugs and the control group did not reach the significance level of $p < 0.05$ (data not shown).

Discussion

The principal findings of the present study were that the comet tail length was significantly longer in the Japanese nurses than in the Japanese control subjects, but also that the subgroup of 57 nurses that had recently handled antineoplastic drugs showed a longer tail length as compared to the control group. These results are in accordance with previous studies performed with the use of the comet assay in Croatia²⁴ and Turkey²⁵. The comet assay has been applied for the detection of early biological effects of DNA-damaging agents such as radiation, chemotherapeutic agents, and active oxygen radicals, in environmental and occupational settings^{20, 21}. In addition, there is evidence of genotoxic, mutagenic, and carcinogenic effects in nurses and pharmacy technicians handling antineoplastic drugs in Western countries¹⁻¹¹. Therefore, our findings suggest that occupational exposures to such drugs may induce subtle DNA damage in Japanese nurses.

On the other hand, we could not find any significant difference in comet tail length or tail moment between two subgroups of nurses, divided according to whether or not the nurses had handled antineoplastic drugs for at least six months, whereas Yoshida *et al.*¹⁷ mentioned that 19 nurses working at the respiratory and blood departments of a hospital had a longer tail length than 18 nurses working at the cardiovascular department ($p = 0.001$). Three explanations for this disagreement are possible as follows: (1) As a group, the nurses of our study were about seven years older than those of Yoshida *et al.* (mean, 30 yr), but also the SD of age was larger in our study. Since aging affects DNA repair activity²¹, as well as the immune system and physical homeostasis, the age distribution may be related to the variability of data; that is, it is possible that the statistical significance

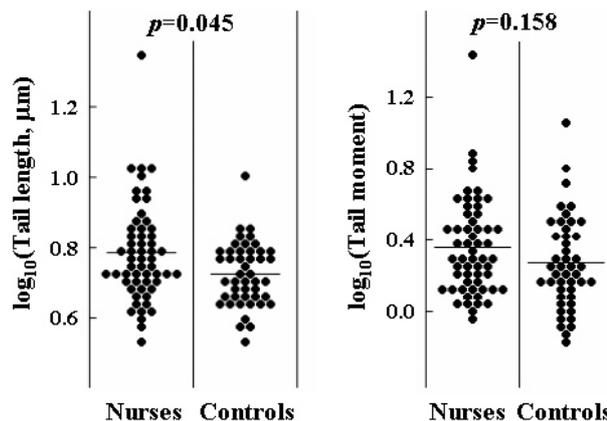


Fig. 2. Tail length and tail moment of comets in peripheral lymphocytes of 57 Japanese nurses who had handled antineoplastic drugs for at least six months and 46 control subjects after adjusting for age, smoking and coffee-drinking habits, hemoglobin, experience of irradiation, and hospital. Transverse lines represent the mean values of each group.

disappeared because of the large variability of our data. (2) The nurses of this study had been periodically rotated through various clinics of individual hospitals. Undoubtedly, 64 of the nurses had not been engaged in handling antineoplastic drugs in the last six months, but some of them might have had prior exposure to such drugs. (3) A nurse who usually handles antineoplastic drugs tends to frequently use personal protective gloves, mask or gown, but a nurse who rarely handles such drugs may touch them or an accidental spill without protection, especially with regard to the handling of linen and other laundry items contaminated with urine, stool, and other excreta of a person receiving hazardous drugs²⁶. For this reason, additional study is required to compare comet assay parameters between nurses from two entirely different hospitals, such as a municipal cancer center and a municipal cardiovascular center.

Concerning comet tail moment, there was no significant difference between the nurses and control subjects (Fig. 2), although the tail moment tended to be larger in the nurses than in the controls. Similarly, Ursini *et al.*²⁷ failed

to find any significant difference in tail moment between healthcare workers and controls. In contrast, Kopjar and Garaj-Vrhovac showed that the tail moments in medical personnel handling antineoplastic drugs differed significantly from those in controls²⁴. Also, two *in vitro* experimental studies observed a significant dose-response relation of tail moment in assessing DNA damage by the comet assay in irradiated human lymphocytes^{28,29}. Taken together, comet tail moment may be less sensitive to antineoplastic drugs than tail length, inasmuch as calculation of the former requires more variables than that of the latter (see *Methods*).

One limitation of the present study is that exposure levels of antineoplastic drugs dispensed at the three hospitals were not evaluated. It would be substantially difficult to monitor the exposure or spill levels of all antineoplastic drugs in the work environment because many kinds of drugs are used there. However, the control subjects of our study seemed to be insulated from the clinical departments of the same hospitals. Cigarette smoking has been identified as one of the main factors affecting DNA damage in nurses handling cytostatic drugs^{30,31}. Age, gender, air pollution, diet, infection, sunlight, Hb, and residential radon have also been regarded as other possible factors affecting the level of DNA damage detected in the comet assay^{21,32}, but there is no evidence to suggest DNA damage due to shift work. Actually, we used most of these confounders, as well as hospital, in the data analysis, and one trained examiner conducted all biochemical tests under masking to avoid a measurement bias. Therefore, it is suggested that our findings were not heavily influenced by any confounding or measurement bias.

Schernhammer *et al.*^{33,34} observed a significantly increased risk of breast or colorectal cancer among women who had worked 1–14 yr, or 15 yr or more on rotating night shifts in a nurses' health study in the US. The authors tried to explain the pathogenesis of breast cancer as exposure to light during night work, *i.e.*, suppressed melatonin production³⁵. These findings should probably be correct, but whether the increased risk in the nurses resulted from occupational hazards associated with handling antineoplastic drugs or from shift work needs to be addressed because the US Department of Commerce estimates that nearly six million healthcare workers handle such hazardous drugs³⁶. Investigators who address work-related cancer factors in healthcare personnel should not overlook this crucial confounder in study design, for doing so may undermine credibility.

In conclusion, the implication of our study is that Japanese nurses who have worked in hospitals using antineoplastic drugs may have a potential risk of DNA damage. The main routes of occupational exposure for the personnel handling hazardous drugs seem to be inhalation and percutaneous absorption because

contamination may occur during the reconstitution of parenteral antineoplastics, during the normal process of excess-drug disposal or as a result of vial leakage, or accidental spill^{14,26}. To minimize the risk, use of biological safety cabinet and appropriate protective equipments, in addition to staff education and training, should be implemented in the healthcare environment. Whether the Ministry of Health, Labour and Welfare needs to present work practice guidelines for personnel dealing with antineoplastic drugs, like the OSHA¹⁴, should also be addressed.

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