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Short Communication

Assessment of Genotoxic Damage in Nurses Occupationally Exposed to Anaesthetic Gases or Antineoplastic Drugs by the Comet Assay

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The potential mutagenic/carcinogenic action of waste anaesthetic gases and antineoplastic drugs in occupationally exposed human populations has been previously reported in several studies[1–3]. Antineoplastic agents discovered in the first two decades of cancer therapy (1950 to 1970) largely interact with DNA or precursors, inhibiting the synthesis of DNA or causing irreparable damage to DNA itself[4]. Considering the mechanisms of the antineoplastic drugs that are used, it is not surprising that many persons involved in health care, especially nurses, are worried about the health effects of these drugs.

Experimental and epidemiological studies suggest that genotoxic effects can arise from inhalation anaesthetics. Due to their widespread use in operating rooms, there is a great concern that operating room personnel as well as patients might be at health risk from anaesthetics[5].

The aim of the present study was to assess the possible genotoxic risk, by the alkaline comet assay, in the peripheral blood lymphocytes of nurses who are handling antineoplastic drugs or are exposed to waste anaesthetic gases.

Materials and Methods

Samples

The study was approved by our local ethics committee and written informed consent was obtained from the subjects after the aim of the study was fully explained.

Fifty-five blood donors, 19 healthy unexposed office workers, 17 nurses who were exposed to waste anaesthetic gases and 19 nurses who were handling antineoplastic drugs participated in the study. We used our previous control data[6] of 16 subjects and included 3 new healthy age and sex matched controls in the present study. The nurses had been exposed to waste anaesthetic gases or antineoplastic drugs for at least 3 mo without interruption (excluding weekends) and were currently employed under similar working conditions for at least 7 yr with similar lifestyles in oncology and anaesthesia departments of different hospitals in the city of Ankara, Turkey.

All the operating rooms had active waste gas scavenging systems. The most commonly used anaesthetics were nitrous oxide, isoflurane, sevoflurane and desflurane. Antineoplastic drug handlers were using gloves and masks during the preparation of the drugs under a vertical flow safety cabinet for the last two years. Cyclophosphamide, cisplatin, 5 fluorouracil, etoposide, vinblastine, vincristine, bleomycine and doxorubicin were the most commonly used antineoplastic drugs in the oncology department. The blood samples from the nurses were taken on Friday's.

Comet assay

The standard protocol for the alkaline comet assay was followed for the lymphocytes of the subjects with minor modifications[7].

Slide scoring

One hundred cells were analyzed using double slides per subject at 400× magnification, under a fluorescence microscope (Zeiss, Germany). In this study each of 100 cells was assigned into 3 categories, NM (no migration), LM (low migration) and HM (high migration), depending on the fraction of DNA pulled out into the tail under the influence of the electric field. The total comet score (TCS) per subject was calculated as; 0×NM (number of comets in category NM) + 1×LM (number of comets in category LM) + 2×HM (number of comets in category HM) as described by Collins[8]. The overall score for each slide was therefore between 0 (undamaged), and 200 (maximal damage). Analysis was performed blind by one slide reader without knowledge of the subjects in order to minimize variability due to subjective scorings.

Statistical analysis

The data were analyzed using the SPSS 16.0 program for Windows. The differences among groups were evaluated by ANOVA with *post-hoc* Tukey test. The Chi-square test was used for categorical comparisons. Variables were compared by using the Bonferroni-corrected Mann Whitney U test within the groups. Correlation analysis was performed by using Pearson's or Spearman's correlation test. $p<0.05$ was considered statistically significant.

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Table 1. General characteristics of exposed groups and controls. Values are mean ± SD (min-max) or n (%)

	Controls (n=19)	Anaesthesia nurses (n=17)	Nurses handling antineoplastic drugs (n=19)
Age (yr)	33.5 ± 5.1 (26–48)	32.6 ± 5.3 (25–45)	32.3 ± 5.9 (25–45)
Sex (female/male)	17 (89.5) / 2 (10.5)	15 (89.5) / 2 (10.5)	17 (89.5) / 2 (10.5)
Smoking habits (nonsmoker/smoker)	7 (36.8) /12 (63.1)	7 (41.2) /10 (58.8)	7 (36.8) / 12 (63.2)
Duration of exposure (yr)	–	11.6 ± 4.2 (7–22)	11.3 ± 4.2 (7–20)

$p>0.05$, ANOVA with *post-hoc* Tukey test or the Chi-square test were used.

Table 2. Total comet scores (TCS)* of exposed groups and controls. Values are mean ± SD.

TCS*	Controls	Anaesthesia nurses (n=17)	Nurses handling antineoplastic drugs (n=19)
Smokers	6.91 ± 3.47 (n=12)	19.7 ± 5.79[a] (n=10)	19.25 ± 4.93[a] (n=12)
Non-smokers	6.71 ± 2.81 (n=7)	17.0 ± 3.51[b] (n=7)	21.0 ± 4.83[c] (n=7)
Total	6.84 ± 3.16	18.58 ± 5.03[d]	19.89 ± 4.84[d]

*TCS= 0×NM (number of comets in category NM) + 1×LM (number of comets in category LM) + 2×HM (number of comets in category HM). [a]: $p<0.001$ (compared to smokers in control group); [b]: $p<0.01$ (compared to non-smokers in control group), [c]: $p<0.001$ (compared to non-smokers in control group); [d]: $p<0.001$ (compared to control group). ANOVA with *post-hoc* Tukey test or the Bonferroni-corrected Mann Whitney U test.

Results

There were no significant differences in individual factors among the three groups ($p>0.05$) (Table 1). Statistically significant differences were detected between the control and exposed groups in terms of mean TCS ($p<0.001$) (Table 2). However, there were no statistically significant differences between the anaesthesia nurses and the nurses handling antineoplastic drugs ($p>0.05$). There were no statistically significant correlations between DNA damage and years of employment in both exposed groups. Comet photographs representing no, low and high migration from an occupationally exposed subject are presented in Fig. 1.

Discussion

In accordance with the most of the studies performed with the comet assay, our findings suggest that occupational exposures to antineoplastic drugs may induce DNA damage in nurses. Laffon *et al*. observed an increase in both the comet assay and the micronucleus (MN) test in nurses handling antineoplastic drugs, although statistical significance was only seen in the comet assay[9]. In agreement with these results, Maluf & Erdtmann found no statistical significance between nurses handling antineoplastic drugs and controls in terms of MN, whereas the DNA damage detected by the comet assay was significantly higher in the exposed group[10]. Recently, Sasaki *et al*. reported increased levels of DNA damage as detected by the comet assay in Japanese nurses handling antineoplastic drugs[11]. Previous studies in Turkish nurses handling antineoplastic drugs reported potential risk[12–14]. In the present study, antineoplastic drug handlers were using gloves and masks during the preparation of the drugs under a vertical flow safety cabinet for the last two years. This period might be too short for a total recovery in DNA damage which is also affected by endogenous (e.g. genetic factors) and exogenous (e.g. antioxidant pool) factors. In 1991, we observed a significantly increased frequency of sister chromatid exchange (SCE) in 23 oncology nurses as compared to a group of unexposed controls[13]. Although the exposure levels were not evaluated, the present study demonstrates that the nurses handling antineoplastic drugs are still at occupational health risk.

Epidemiological studies have presented a controversial picture about the occupational health risk of anaesthetics. Rozgaj *et al*. noted significantly increased frequencies of chromosomal aberrations (CAs) and MN while increased SCE frequency was not significant in personnel exposed to anaesthetic gases[15]. Wiesner *et al*. found a higher MN level in anaesthesiologists and nurses exposed to high levels of halothane and isoflurane compared with a control group[1]. Chandrasekhar *et al*. reported a

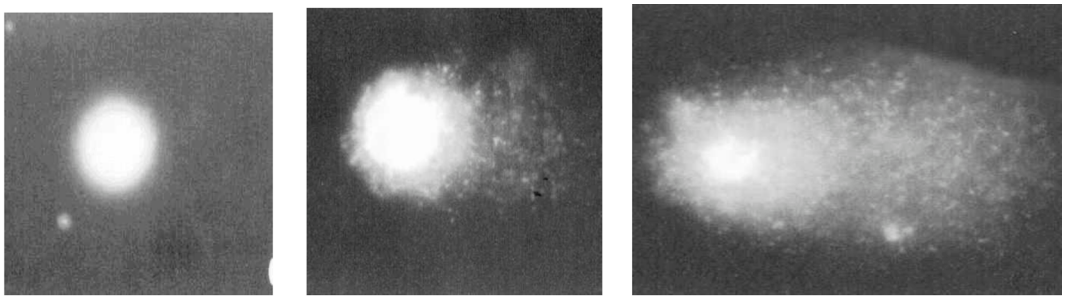

Fig. 1. Representative comet assays of cells with a) no migration, b) low migration, and c) high migration.

statistically significant increase in DNA damage as shown by CA, MN and the comet assay in operating room personnel exposed to anaesthetic gases[16]. Although we couldn't evaluate the exposure levels to anaesthetic gases, our study points to an increase in DNA damage in anaesthesia nurses working in operating rooms with artificial ventilation and active scavenging systems.

Using the comet assay, we previously demonstrated an elevated grade of DNA damage in operating room personnel exposed to waste anaesthetic gases in Ankara in 1998[17]. Recently, we examined the genoprotective role of Vit E and Vit C supplementation in technical anaesthesiology staff working in operating theatres and found significantly higher levels of genotoxic damage before antioxidant treatment[6]. In our previous study, none of the operating theatres had active waste gas scavenging systems and the DNA breakage observed in the lymphocytes of the anaesthesiology staff was higher than that of the nurses in the present study. Although the working conditions were better in this study, significant differences were detected between the control and the anaesthesia nurses in terms of their mean total comet scores. Contamination of operating theaters with anaesthetic gases is inevitable due to the probability of leaks from anaesthesia systems, release of gases through expiration by patients, or in pediatric patients, anaesthesia induction with masks, open anaesthesia circuits, and the use of endotracheal tubes without cuffs. Even in operating theaters with proper ventilation and active waste gas scavenging systems, the exposure levels recommended by NIOSH may not be achieved[18].

Our study as well as the majority of previously published human biomonitoring studies failed to show an effect of smoking on DNA migration in the comet assay, while some studies indicate such an effect[19]. Various explanations for the reported discrepancies have been proposed, including the power of the statistical analysis and also seasonal and regional differences. It is suggested that the major problems are the types of the damage detected by the alkaline comet assay which are DNA strand breaks, alkali-labile sites and incomplete excision repair sites. However, smoking is known to induce DNA adducts that do not have a strong effect on comet assay parameters[20]. Considering the factors discussed above, nonsignificant comet results between smokers and nonsmokers in our study can mainly be explained by the small sample size, low statistical power, and the cigarette consumption of smokers (none of them were heavy smokers, ≤20 cigarettes/day).

In conclusion, this study points to the potential risk of DNA damage in nurses who are handling antineoplastic drugs or are exposed to waste anaesthetic gases. Although more research is needed to clarify the genotoxic risk due to these occupational hazards, it is clear that further minimizing the exposure is needed in the studied hospitals. Precautions will help to reduce the health risks of occupationally exposed personnel in the hospitals. Further study should include a better design using a larger exposure group with measurement of the concentrations of the hazardous agents.

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References

1) Wiesner G, Hoerauf K, Schroegendorfer K, Sobczynski P, Harth M, Reudiger HW. High-level, but not low-level, occupational exposure to inhaled anaesthetics is associated with genotoxicity in the micronucleus assay. Anesth Analg 2001; 92: 118–22.
2) Kopjar N, Garaj-Vrhovac V. Application of the alkaline comet assay in human biomonitoring for genotoxicity: A study on Croatian medical personnel handling antineoplastic drugs. Mutagenesis 2001; 16: 71–8.
3) Sharbel WM, Erdtmann B. 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 2000; 471: 21–7.
4) Calabresi P, Chabner BA. Chemotherapy of neoplastic

disease. In: . Hardman JG, Limbird LE, editors. Goodman and Gilman's: The pharmacological basis of therapeutics. New York: The McGraw-Hill Companies; 2001. p. 1381–461.
5) Buring JE, Hennekens CH, Mayrent SL, Rosner B, Greenberg ER, Colton T. Health experiences of operating room personnel. Anaesthesiology 1985; 62: 325–30.
6) Sardas S, Izdes S, Ozcagli E, Kanbak O, Kadioglu E. The role of antioxidant supplementation in occupational exposure to waste anaestetic gases. Int Arch Occup Environ Health 2006; 80: 154–9.
7) Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184–91.
8) Collins AR. The comet assay. Principles, applications, and limitations. Meth Mol Biol 2002; 203: 163–77.
9) Laffon B, Teixeira JP, Silva S, *et al*. Genotoxic effects in a population of nurses handling antineoplastic drugs, and relationship with genetic polymorphisms in DNA repair enzymes. Am J Ind Med 2005; 48: 128–36.
10) Maluf SW, Erdtmann B. 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 2000; 471: 21–7.
11) Sasaki M, Dakeishi M, Hoshi S, Ishii N, Murata K. Assessment of DNA damage in Japanese nurses handling antineoplastic drugs by the comet assay. J Occup Health 2008; 50: 7–12.
12) Burgaz S, Özdamar YN, Karakaya AE. A signal assay for the detection of genotoxic compounds: Application on the urines of cancer patients on chemotherapy and of nurses handling cytotoxic drugs. Human Toxicol 1988; 7: 557–60.
13) Sardas S, Gök S, Karakaya AE. Sister chromatid exchange in lymphocytes of nurses handling antineoplastic drugs. Toxicol Lett 1991; 55: 311–5.
14) Undeger U, Basaran N, Kars A, Guc D. Assesment of DNA damage in nurses handling antineoplastic drugs by alkaline comet assay. Mutat Res 1999; 439: 277–85.
15) Rozgaj R, Kasuba V, Jazbec A. Preliminary study of cytogenetic damage in personnel exposed to anaesthetic gases. Mutagenesis 2001; 16: 139–43.
16) Chandrasekhar M, Rekhadevi PV, Sailaja N, *et al*. Evaluation of genetic damage in operating room personnel exposed to anaesthetic gases. Mutagenesis 2006; 21: 249–54.
17) Sardas S, Aygün N, Gamli M, Ünal Y, Ünal N, Berk N. Use of alkaline Comet assay (single cell gel electrophoresis technique) to detect DNA damages in lymphocytes of operating room personnel occupationally exposed to anaesthetic gases. Mutat Res 1998; 418: 93–100.
18) Hoerauf K, Funk W, Harth M, Hobbhahn J. Occupational exposure to sevoflurane, halothane and nitrous oxide during paediatric anaesthesia. Waste gas exposure during pediatric anaesthesia. Anaesthesia 1997; 52: 215–9.
19) Moller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidemiol Biomarkers Prev 2000; 9: 1005–15.
20) Speit G, Witton-Davies T, Heepchantree W, Trenz K, Hoffmann H. Investigations on the effect of cigarette smoking in the comet assay. Mutat Res 2003; 542: 33–42.