

CHROMOSOME ABERRATIONS AND MICRONUCLEUS FREQUENCY IN ANAESTHESIOLOGY PERSONNEL

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Occupational exposure to anaesthetic gases is associated with various adverse health effects. Genetic material is a sensitive target of numerous harmful agents. The aim of this study was to examine whether chromosomal damage could serve to indicate exposure to anaesthetics. Twenty-eight anaesthesiologists, 16 technicians, and 32 control subjects were examined for chromosome aberrations and micronucleus frequency. An increase in chromosome damage was found in both exposed groups. Micronucleus frequency increased significantly, showing higher rates in women. The observed differences between the sexes in respect to the exposure risk call for further, targeted investigation.

Key words:
anaesthetics, chromosome damage, genotoxicity,
occupational exposure

Hospital workers employed in operating rooms are chronically exposed to various levels of volatile anaesthetics which can have adverse effects to their health. Numerous studies concerning genotoxic effects of anaesthetics suggest an association between the exposure to anaesthetics and the incidence of damage of genetic material. *Reitz and co-workers* (1, 2) reported the occurrence of DNA single-strand breaks in patients and medical personnel exposed to anaesthetic gases. *Arda^o and co-workers* (3, 4) assessed DNA damage in peripheral lymphocytes of a medical staff exposed to various anaesthetic gases and in patients anaesthetised with isoflurane. Using the alkaline comet assay, they found a significantly increased number of cells showing DNA migration in exposed individuals. *In vitro* experiments corroborated those results (5). Cytogenetic changes have been observed in mammalian cells under conditions of *in vivo* or *in vitro* experiments with different anaesthetics (6, 7). Long-term occupational exposure to anaesthetic gases in hospital workers calls for special concern. Several studies of operating room personnel have identified increased frequency of chromosome aberrations (8–13). *Chang and co-workers* (14) reported an increase in micronucleus frequency, whereas findings related to sister chromatid exchanges were

inconsistent. *Husum and Wulf* (15) and *Bigatti and co-workers* (8) reported negative results, while *Natarajan and Santhiya* (10) and *Karelouva and co-workers* (11) reported an increased sister chromatid exchange frequency in exposed subjects.

The aim of this study was to determine the genotoxic risk of occupational exposure to anaesthetic gases of anaesthesiologists and anaesthesiology technicians. The techniques used were chromosome aberrations analysis and micronucleus test.

SUBJECTS AND METHODS

Study population

The study included three groups: 28 anaesthesiologists, 16 technicians, and 32 controls. The control subjects were selected from non-exposed workers in the same hospital such as clerks and the newly hired employees screened for chromosome aberrations during pre-employment medical tests. Demographic data and information on smoking habits, alcohol consumption, therapy with drugs, recent vaccination, and therapeutic exposure to radiation were collected for all subjects. The mean age of examinees was 31.5 in the control group, 35.1 in technicians, and 43.0 in anaesthesiologists (Table 1). According to answers, all exposed personnel worked in operating rooms without ventilation. Nitrous oxide and halothane were the most commonly used anaesthetics.

Table 1 *Characteristics of examined groups with respect to sex, age, cigarette smoking, and years of exposure to anaesthetic gases*

Group	No. of subjects	Age x (range)	Sex M:F	Years of exposure \bar{x} (range)	Smokers N (%)
Control	32	31.5 (22-49)	15:17	0	9 (28)
Anaesthesiologists	28	43.0 (32-58)	14:14	12.9 (1-32)	11 (39)
Technicians	16	35.1 (27-48)	8:8	11.6 (1-25)	6 (38)

Cytogenetic methods

Heparinised blood samples for chromosome aberration assay and micronucleus assay were collected by venipuncture. Cultures were initiated simultaneously according to standard protocols. One millilitre of whole blood was added to 8 ml of F-10 medium (Gibco, United Kingdom) supplemented with 20% calf serum (Biological Industries, Israel), penicillin (100 U/ml, Pliva, Croatia), streptomycin (100 μ g/ml, Pliva, Croatia), and 1% phytohaemagglutinine (Murex, United Kingdom).

For chromosome aberration analysis, cultures were incubated at 37 °C for 48 h. Colchicine was added to the cultures 3 h before harvesting. Giemsa-stained slides were coded and scored blind under a light microscope.

For the micronucleus assay, 6.0 µg/ml of cytochalasin B (Sigma, Germany) was added to the cultures after a 44-hour incubation. Cells were harvested 72 h after phytohaemagglutinine stimulation.

Two hundred first metaphases per subject were analysed for chromosome aberration frequency and 1,000 binucleated cells per subject were analysed for micronucleus assay. The slides were analysed by two scorers from the same laboratory.

All statistical comparisons applied the analysis of variance (ANOVA) with $P < 0.05$ taken as the level of significance. The control group served as the baseline. Since we found sex a statistically significant confounder in the analysis of micronucleus frequency, we analysed men and women separately.

RESULTS

Anaesthetics induced chromosome damage in the exposed subjects. The influence of age, duration of exposure, or smoking did not show any influence on the incidence of chromosome damages. However, significant differences in the frequency of micronucleated cells were found between men and women. Table 1 shows the distribution of subjects with respect to age, sex, years of exposure, and smoking. The exposed groups matched in sex and duration of exposure to anaesthetics, while no correspondence was possible for age. The age difference reflects longer training of anaesthesiologists than of technicians. Similarly, the control group was dominated by young, newly admitted personnel. The contribution of smokers ranged from 39% in anaesthesiologists to 28% in the control group.

Table 2 shows frequencies of chromosome aberrations (single breaks, double breaks, acentric fragments, and dicentric chromosomes) and the frequency and distribution of micronuclei by profession. Statistically significant increase was observed only for acentric fragments in anaesthesiologists and for dicentrics in technicians. The frequency of micronucleated cells was significantly different between the exposed groups and controls, both for cells with one micronucleus or more micronuclei.

The incidence of micronuclei showed differences between the sexes in the exposed groups, while no such difference was observed in the control group. Table 3 shows the distribution of micronucleus frequency by sex. The frequency of micronucleated cells was significantly higher in the exposed women.

DISCUSSION

The increased frequency of aberrations in the exposed subjects in this study confirmed our previous results (13) and the results of other authors (8, 11, 12). Micronu-

Table 2 Frequency of cytogenetic damages by profession

Group	Chromosome aberrations (mean per 200 metaphases)				Distribution of micronuclei (MN)				MN (%)
	SB	DB	AC	DIC	MN-1	MN-2	MN-3	MN-4	
Control	0.66 ± 0.12	0.41 ± 0.11	0.66 ± 0.14	0.06 ± 0.04	6.94 ± 0.89	0.41 ± 0.14	0.06 ± 0.04	0	7.78 ± 1.14
Anaesthesiologists	0.61 ± 0.17	0.57 ± 0.17	1.64 ± 0.31*	0.21 ± 0.09	20.8 ± 2.50*	4.54 ± 1.15*	0.79 ± 0.23*	0.14 ± 0.07*	33.8 ± 4.64*
Technicians	0.69 ± 0.20	0.50 ± 0.18	1.31 ± 0.57	0.38 ± 0.20*	20.0 ± 3.99*	2.63 ± 0.58*	0.56 ± 0.22*	0.25 ± 0.11*	27.9 ± 4.78*

All data are shown as mean±SEM.

SB – chromatid break; DB – chromosome break; AC – acentric fragment; DIC – dicentric chromosome; MN-1 to MN-4 – binucleated lymphocytes containing one, two, three, or four micronuclei; MN (%) – total number of micronuclei per 1000 binucleated lymphocytes.

*Significantly different to control value (P<0.05)

Table 3 Frequency of micronuclei by sex

Group		No. of cells with micronuclei (MN)				MN%
		MN-1	MN-2	MN-3	MN-4	
Control	M	6.60 ± 1.34	0.53 ± 0.27	0.13 ± 0.09	0	7.73 ± 1.92
	F	7.24 ± 1.21	0.29 ± 0.11	0	0	7.82 ± 1.37
Anaesthesiologists	M	17.8 ± 3.39	2.07 ± 0.65	0.50 ± 0.25	0	23.4 ± 4.54
	F	23.7 ± 3.62	7.00 ± 2.03*	1.07 ± 0.37	0.29 ± 0.13*	42.1 ± 7.46*
Technicians	M	8.88 ± 2.56	1.38 ± 0.56	0.50 ± 0.33	0.25 ± 0.16	14.1 ± 4.37
	F	31.1 ± 5.13*	3.88 ± 0.83*	0.63 ± 0.32	0.25 ± 0.16	41.8 ± 4.95*

All data are shown as mean ± SEM.

MN-1 to MN-4 – binucleated lymphocytes containing one, two, three, or four micronuclei; MN (%) – total number of micronuclei per 1000 binucleated lymphocytes.

*Significantly different to control value (P<0.05)

clei appear to be more sensitive biomarkers of anaesthetic-induced genotoxicity to humans than chromosome aberrations. The number of cells containing one or more micronuclei increased in all exposed groups, which was in agreement with other authors. *Chang and co-workers* (14) reported increased micronuclei formation in nurses occupationally exposed to nitrous oxide. *Robbiano and co-workers* (7) tested six halogenated anaesthetics for their ability to induce micronuclei formation in rat kidney. All except enflurane significantly increased the micronucleus frequency, while halothane and trichloroethylene also reduced the number of binucleated cells, presumably due to their toxicity.

Sex proved to be one of the factors influencing micronucleus frequency in human lymphocytes (16). The difference in micronucleus frequency between the sexes was also evident in our study, as the exposed women showed significantly higher results than the exposed men. *Fenech and co-workers* (17) reported significantly higher spontaneous micronucleus frequency in women and attributed it to the loss of X chromosomes which in turn contribute to the micronucleus yield in women. *Bonassi and co-workers* (18) confirmed higher micronucleus frequency in women and showed that sister chromatid exchange or chromosome aberrations frequency were not able to establish significant difference between the sexes. In contrast, a study of healthy donors by *Di Giorgio and co-workers* (19) did not find any influence of sex on the micronucleus level.

Our study found significant effect of exposure to anaesthetics in women, but not in men. On the other hand, the age-related or smoking-related effects on the occurrence of micronuclei could not be established. In addition to age and sex, dietary factors can affect chromosome aberrations and micronucleus frequency. It has been shown by other authors that exposure to nitrous oxide destroys vitamin B₁₂, which in turn influences the increase in MN frequency (20, 21). This could explain the increase in micronuclei in personnel exposed to nitrous oxide. *Fenech* (22) reported that micronucleus frequency was minimised when the level of plasma homocysteine was below 7.5 µmol/L and plasma B₁₂ was above 300 pmol/L.

To conclude, the results of our study indicate that exposure to anaesthetic gases induced changes on chromosomes. The micronucleus test was more sensitive than chromosome aberrations for evaluating genotoxic effects of anaesthetic gases to humans. The suggested higher risk for women calls for special attention. As this study included a relatively small number of subjects, further investigation should include a larger group of medical professionals exposed to anaesthetics. It is also necessary to control the concentrations of anaesthetics in operating rooms. Finally, considerations should include other indicators, especially plasma levels of folate and vitamin B₁₂.

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*Sažetak***KROMOSOMSKE ABERACIJE I UČESTALOST MIKRONUKLEUSA U ANESTEZIOLOGA I ANESTEZIOLOŠKIH TEHNIČARA**

Dugotrajna profesionalna izloženost niskim dozama plinovitih anestetika može imati različite nepoželjne učinke na zdravlje. Iako se zbog slabe topljivosti u krvi i tkivima brzo uklanjaju iz organizma, poznati su njihovi neurotoksični, hepatotoksični, a prema nekim istraživanjima i karcinogeni učinci. Istraživanja genotoksičnosti anestetika dala su različite rezultate. Citogenetske metode pokazale su se vrlo prikladnim pri istraživanjima na populaciji profesionalno izloženih osoba.

Da bi se procijenio rizik od profesionalne izloženosti anestetima u skupinama anesteziologa i anestezioloških tehničara analizirane su kromosomske aberacije i učestalost mikronukleusa. Istraživanje je obuhvatilo 28 anesteziologa i 16 anestezioloških tehničara pretežno izloženih dušičnom oksidu i halotanu. U kontrolnoj skupini analizirane su 32 osobe raznih profesija koje na svojim radnim mjestima nisu bile izložene fizikalnim ni kemijskim mutagenima. Skupine ispitanika usklađene su po spolu i duljini izloženosti anestetima. Dob ispitanika nije mogla biti potpuno usklađena zbog različite dužine školovanja ispitanika, a u kontrolnoj skupini i zbog odabira mladih, novozaposlenih osoba kako bi se izbjegla profesionalna izloženost mutagenima.

U izloženim skupinama nije uočen utjecaj dobi, dužine izloženosti i pušenja na učestalost kromosomskih oštećenja. Porast broja kromosomskih oštećenja nađen je u obje izložene skupine u odnosu na kontrolu. Statistički značajan porast acentričnih fragmenata nađen je kod anesteziologa, a dicentrika kod anestezioloških tehničara. Učestalost mikronukleusa u binuklearnim limfocitima bila je značajno povišena u obje izložene skupine. Ovaj test pokazao se osjetljivijim od analize kromosomskih aberacija, pokazujući i statistički značajno povišenje u učestalosti oštećenja kod žena izloženih anestetima.

Ključne riječi:

anestetiци, genotoksičnost, kromosomska oštećenja, profesionalna izloženost

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