Genotoxic effects of anesthetics in operating room personnel evaluated by micronucleus test

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Abstract
Exposure to certain chemical agents causes damage to the genetic material. There is controversy about the genotoxic and/or mutagenic effect caused by acute or chronic anesthetics exposure. The aim of this research was to assess and compare the frequency of micronuclei (MN) in professionals working in operating rooms and other hospital areas. The genotoxic and cytotoxic effects were evaluated in lymphocytes using the micronucleus test and the proliferative index, respectively. We examined peripheral blood lymphocyte cultures from 30 operating room professionals (exposed group) and 30 professionals non-exposed in other hospital areas of the same hospital (control group). There was no statistically significant difference in proliferative index between the groups. Nevertheless, there was an increase in MN frequency in binucleated (BN) cells in the exposed group (p=0.0003) compared to the control group. Moreover, there was a statistically significant difference between genders (p=0.0187), and the frequency of MN in BN cells from women was higher than in men. Therefore, gender influenced the frequency of MN. The age and period of working time in an operating room influenced the MN frequency only in women professionals. Thereby, there was a genotoxic effect in occupationally exposed professionals, and the micronucleus test could be used in the biomonitoring of human populations for evaluating the risk of developing cancer.

Keywords: Anesthetics, micronuclei test, genotoxic, occupational exposure

Introduction
Volatile anesthetics are the major pollutants in operating rooms, where the professionals are exposed to low doses of them, for long periods of time. Neurotoxic [32], hepatotoxic [22], nephrototoxic [24,32] and carcinogenic effects [13,32,39], as well as fertility alterations, increased incidence of spontaneous abortions and congenital abnormalities have been observed in these professionals [3,24,41,45,46].

Nevertheless, there is still some controversy regarding the genotoxic and/or mutagenic effects of acute or chronic exposure to anesthetic gases [14,25,27], even though significant increases in the frequency of chromosome aberrations and of micronuclei (MN) in peripheral blood lymphocytes of professionals exposed to such agents have been reported [8,11,12,29,31,35,42,44].

The micronucleus test is a method for the assessment of several kinds of cytogenetic damages, used in the biomonitoring of human populations for evaluating the risk of developing cancer and identifying the genotoxic potential of chemical, physical and biological agents [1,31,35,42].

Originally, the micronucleus test focused exclusively on the binucleated (BN) cells [16]. However, it was observed that MN in mononucleated (MO) cells, as well as proliferative index (PI), could provide complementary information, since these data may indicate damage that is present in vivo, before cell culture is set up, which makes them interesting for the purpose of biomonitoring [28,40].

Information about possible genetic damage in professionals following exposure to waste anesthetic gases in operating rooms in Brazil is scant. Therefore, the objective of the present study was to verify the frequency of MN in professionals who were occupationally exposed to anesthetic gases in operating rooms and compare with professionals non-exposed in other hospital areas of the same hospital.

Material and methods
Population study
Participation in the study took place after the signing of a Free and Informed Consent Form. The study was conducted following the approval of the Research Ethics Committee of the Federal University of the Triângulo Mineiro (CEP/UFTM – n° 962). The sample was composed of 60 professionals who worked at the Clinics’s Hospital/UFTM, located at Uberaba, State of Minas Gerais, Brazil and were recruited between the years 2007 and 2008. They were divided into two groups: exposed group, comprising 30 professionals who worked in operating rooms; and control group, comprising another 30 professionals, who did not work in operating rooms. The groups were matched for age and gender. Table 1 shows the main characteristics of both groups.

Each individual was interviewed using a standard questionnaire with questions regarding age, gender, use of medications, medical treatments, life style (consumption of cigarettes and...
alcohol), and occupational questions (time in the profession, daily working hours, exposure to X-rays). The selected professionals had been working at the hospital for at least two year. The professionals who worked in operating rooms spent 10h/day in the operation room and worked for 6 days/week. During their service as operating room personnel, all subjects were exposed to a complex mixture of anesthetic agents (halothane, enflurane, isoflurane, sevoflurane, nitrous oxide among others). It was assured that the operating room personnel and the controls did not statistically differ from each other except for occupational exposure. Professionals who had undergone medical treatment of any kind, had any infectious disease or worked in a service with exposure to X-rays were excluded of sample.

A code number was assigned to each individual sample and its corresponding slides for culture identification and identity preservation.

### Micronucleus test

Samples of whole blood were drawn from all subjects, and conventional short-term lymphocyte cultures were made. Cultures were set up by adding 0.5 ml whole blood to 4.5 ml of RPMI medium with 20% fetal calf serum (Gibco®) and antibiotics (penicillin and streptomycin). Lymphocytes were stimulated with 0.18 mg/ml phytohemagglutinin (Sigma®) and incubated at 37°C for 72h. Cytochalasin B (Sigma®) at a final concentration of 6µl/ml was added at 44h of incubation. The cultures were harvested and the cells were submitted to a mild hypotonic treatment and then fixed with methanol:acetic acid (3:1). Microscope slides were prepared and, after the cells dried, the slides were stained with 4% Giemsa for 5 minutes. The slides were coded at the time of preparation and scoring. The frequency of MN was determined in 2000 BN and in 2000 MO lymphocytes per culture [18,21,28,40]. The slides were analyzed using a Carl Zeiss® microscope with an X400 objective. Scoring criteria for selection of BN cells and MN were set according to the criteria of Fenech and Morley [16]. The slides were decoded before statistical analysis for comparison. As a parameter for cytotoxicity, nuclear division index (NDI) was calculated according to the formula: NDI = (M1+2M2+3M3+4M4)/N, where M1–M4 represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells) [21].

### Table 1. Demographic characteristics of study population.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposed</th>
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<tbody>
<tr>
<td><strong>Sex n° (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (46.7)</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (53.3)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40.50±12.06</td>
<td>40.28±11.06</td>
</tr>
<tr>
<td>Range</td>
<td>28-61</td>
<td>26-60</td>
</tr>
<tr>
<td>Female</td>
<td>41.50±11.63</td>
<td>41.75±11.83</td>
</tr>
<tr>
<td>Range</td>
<td>23-61</td>
<td>25-60</td>
</tr>
<tr>
<td><strong>Years of Experience</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>--</td>
<td>13.83±10.93</td>
</tr>
<tr>
<td>Range</td>
<td>--</td>
<td>2-37</td>
</tr>
</tbody>
</table>

Cultures were set up by adding 0.5 ml whole blood to 4.5 ml of RPMI medium with 20% fetal calf serum (Gibco®) and antibiotics (penicillin and streptomycin). Lymphocytes were stimulated with 0.18 mg/ml phytohemagglutinin (Sigma®) and incubated at 37°C for 72h. Cytochalasin B (Sigma®) at a final concentration of 6µl/ml was added at 44h of incubation. The cultures were harvested and the cells were submitted to a mild hypotonic treatment and then fixed with methanol:acetic acid (3:1). Microscope slides were prepared and, after the cells dried, the slides were stained with 4% Giemsa for 5 minutes. The slides were coded at the time of preparation and scoring. The frequency of MN was determined in 2000 BN and in 2000 MO lymphocytes per culture [18,21,28,40]. The slides were analyzed using a Carl Zeiss® microscope with an X400 objective. Scoring criteria for selection of BN cells and MN were set according to the criteria of Fenech and Morley [16]. The slides were decoded before statistical analysis for comparison. As a parameter for cytotoxicity, nuclear division index (NDI) was calculated according to the formula: NDI = (M1+2M2+3M3+4M4)/N, where M1–M4 represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells) [21].

### Statistical analysis

Statistical analysis was performed using the StatView 6.0 software. The level of significance was set at p<0.05. Normality of the data was determined by means of the Kolmogorov-Smirnov test. Multifactorial variance analysis (ANOVA– FACTORIAL) was made for gender and condition (control or exposed) and micronuclei frequency. Student’s t-test and Fisher’s PLSD test were employed for analyzing the correlation between exposure time and frequency, and between age and MN frequency, and descriptive statistics was performed. We could not estimate the interaction between time of exposure and age.

### Results

As mentioned above, the sample was composed of 32 women (53.3%) and 28 men (46.7%) professionals, 30 of which worked in operating rooms (exposed) and 30 of which worked in other hospital areas, matched for gender and age.

With regard to the NDI, the mean and standard deviation in the exposed and control group were 1.68±0.25 and 1.69±0.21, respectively (p=0.9681). This data indicated that occupational exposure to the anesthetic gases did not have toxic effects on lymphocytes impairing their mitotic proliferation in vitro. Considering the total sample, a statistically significant increase of MN frequency was observed only in the BN cells (p=0.0003) of the exposed group compared with control group (Figure 1). MN frequency in BN cells was increased in women (p=0.0023) as well as in men (p=0.0001) compared with the respective control group. On the other hand, an increased MN frequency in MO cells was found only in males (p=0.0035) (Table 2). Only in the control group, the women showed statistically
significant increase of MN frequency in BN cells (p=0.0132) as well as in MO cells (p=0.0243) compared to men (Table 3 and 4 respectively).

To analyze the effect of age in exposed and control groups, they were divided into two subgroups. One of 25-44 years of age was composed of 16 individuals (8 men and 8 women) and the other with 45-64 years of age was composed of 14 individuals (6 men and 8 women).

Performing the analysis separating by age and gender, a statistically significant increase in MN frequency of BN cells (p=0.0067) and MO cells (p=0.0398) was observed with growing age only in women of the exposed group (Figures 2 and 3 respectively).

The incidence of MN in MO and BN cells (Figure 4) increased with time of exposure to anesthetics only in women.

Discussion

Among the most promising prevention strategies are the use and validation of biomarkers, which can anticipate the clinical diagnosis and steer measures of prevention in populations under risk, resulting from exposure to carcinogens or to a genetic susceptibility [26,34].

The use of MN as biomarkers is based on the fact that cancer cells present cytogenetic abnormalities, which reinforces the hypothesis that chromosomal damages are directly involved in cancer etiology [50].

Several factors can influence the frequency of MN in the cell, such as age [2,16,19,33,36,47], gender [5,6,20,19], vitamins, medical treatment, daily exposure to genotoxic agents, among others [7,20].

Table 2. Descriptive measures of the number of MN (MN nº/1000 BN cells and MN nº/1000 MO cells) in the control and exposed groups by gender and result of ANOVA test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Exposed</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>BN cells</td>
<td>16</td>
<td>16</td>
<td>0.0203*</td>
</tr>
<tr>
<td>Male</td>
<td>BN cells</td>
<td>14</td>
<td>14</td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>MO cells</td>
<td>16</td>
<td>16</td>
<td>0.9503</td>
</tr>
<tr>
<td>Male</td>
<td>MO cells</td>
<td>14</td>
<td>14</td>
<td>0.0035*</td>
</tr>
</tbody>
</table>

*significant difference (p <0.05), mean±SD = mean±standard deviation, n= number of individuals.

Table 3. Descriptive measures of the number of MN (MN Nº/1000 BN cells) in the control and exposed groups between gender and result of ANOVA test.

<table>
<thead>
<tr>
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<th>Parameter</th>
<th>Exposed</th>
<th>Control</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>BN cells</td>
<td>16</td>
<td>16</td>
<td>0.0243*</td>
</tr>
<tr>
<td>Male</td>
<td>BN cells</td>
<td>14</td>
<td>14</td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>MO cells</td>
<td>16</td>
<td>16</td>
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</tr>
<tr>
<td>Male</td>
<td>MO cells</td>
<td>14</td>
<td>14</td>
<td>0.0035*</td>
</tr>
</tbody>
</table>

*significant difference (p <0.05), mean±SD = mean±standard deviation, n= number of individuals.

Table 4. Descriptive measures of the number of MN (MN Nº/1000 MO cells) in the control and exposed groups between gender and result of ANOVA test.

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Exposed</th>
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<tbody>
<tr>
<td></td>
<td>Gender</td>
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<td>Female</td>
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<td>16</td>
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<td>0.0243*</td>
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<tr>
<td>Male</td>
<td>BN cells</td>
<td>14</td>
<td>14</td>
<td>0.0001*</td>
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<tr>
<td></td>
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<td>16</td>
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<td>Male</td>
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<td>14</td>
<td>0.0035*</td>
</tr>
</tbody>
</table>

*significant difference (p <0.05), mean±SD = mean±standard deviation, n= number of individuals.
The increase in MN frequency indicates defects in DNA repair and in chromosome segregation, which might result in the generation of daughter cells with an altered gene dosage and/or dysregulated gene expression, thus leading to a chromosomal instability phenotype, as observed in cancers [9,15,18,34]. The study of Leach and Jackson-Cook [2001] [30] showed that any chromosome could be present in MN.

The mechanism by which the anesthetics induce DNA damage is still controversial. In the present study, we observed a significant increase in MN frequency in the exposed group, in both genders. There are previous reports in the literature showing a significant increase in MN frequency in exposed women [31,37,43]. In our study, however, we also found a difference in the man professionals.

In this study, the women showed statistically significant increase of MN frequency when compared to men. Could not be determined the reason for the high frequency of MN in women, but one hypothesis is the loss of chromosome X, which is prone to suffer spontaneous as well as induced malsegregation, especially in older women [17,23].

Data from the literature showed that the increase in MN frequency in BN cells with age was observed in both genders. [4,6,10,16,17,19,38,51]. However, in the present study, we found an increase only in the exposed women, probably because the number of individuals in our sample was too small to separate them by age bracket.

The relationship of age with a decline in the efficiency of the DNA repair processes and the accumulation of mutations due to adverse endogenous and exogenous conditions results in an increasing level of DNA damage, reflected on the cytogenetic level by an increase in the frequency of chromosome aberrations [4,38,48,51] and MN in the peripheral blood lymphocytes of elderly individuals [6,7,16,17].

Bukvic et al., (2001) [10] showed an increase in MN frequency in older individuals, and this was observed more frequently in females. Several studies have shown an association between chronological age and aneuploidy, and the most frequent loss of the X and Y chromosomes in females and males respectively [4,5,38].

The result of our study indicates the danger of exposure to anesthetic gases in exposed workers, suggesting the need to reduce the period of exposure. The anesthetic gases scavenger and air conditioning equipment should be used and sufficient ventilation should be supplied. Furthermore, preventive health examination of all exposed workers should be periodically performed.

Although the chromosome aberration analysis is more sensitive, it requires time and highly skilled professionals, which makes the biomonitoring of large groups of workers difficult. Therefore, MN analysis in human lymphocytes using the cytochalasin B technique [16] has been proposed as a valid and less laborious alternative for large-scale studies [15].

Since chromosome instability can occur by chromosome loss or break and the evaluation of micronuclei furnishes a measurement of both chromosome losses and breaks, it therefore plays an important role as a biomarker for cancer risk. Thus, the micronucleus test could be used in the planning, implementation and validation of cancer vigilance and prevention policies for professionals who are occupationally exposed to anesthetics.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MB and RLS substantially contributed to the conception and design. TKA, RLS, CWP, RMC, FBB, NCR and MB acquired and interpreted the data. TKA, RLS, FBB, NCR, CWP and MB drafted the article or revised it critically for important intellectual content. TKA, RLS, FBB, NCR, CWP, RMC and MB finally approved the version to be published.

Acknowledgement
We would like to thank the volunteers for their helpful cooperation. This work was supported by the FAPEMIG and FUNEPU.

Publication history
Received: 07-Feb-2013 Revised: 13-Mar-2013
Re-Revised: 12-May-2013 Accepted: 06-Jun-2013
Published: 03-Aug-2013

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Citation:
http://dx.doi.org/10.7243/2049-9752-2-26