Cytogenetic Damage in Operating Room Nurses Exposed to Anesthetic Gases

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Abstract

Background: Although eliminated rapidly from the body due to low solubility in blood and tissues, anesthetic gases have been reported to be neurotoxic, teratogenic and carcinogenic. Genetic material has been shown to be a sensitive target of numerous harmful agents.

Aim of the Work: To evaluate genotoxic risk of occupational exposure to anesthetic gases in a group of operating room nurses.

Subjects and Methods: A group of 27 operating room nurses exposed to waste anesthetic gases and 18 control nurses were examined for chromosome aberrations and sister chromatid exchanges in peripheral blood lymphocytes. The exposed group was compared with the control group which was matched by age, socioeconomic level and duration of employment.

Results: The prevalence of all neurological manifestations were higher in exposed nurses compared to control group. A significant increase in chromosome damage in exposed nurses as detected by total chromosomal aberrations, gaps, deletion and endomitosis while the increase in centromere separation and chromatid breaks was not significant. There was an increase in sister chromatid exchange frequency in exposed nurses compared to control even it was not significant. Most of these parameters of genetic damage in exposed nurses were positively correlated with age and duration of exposure to inhaled anesthetics.

Conclusion: The results of our study suggest that exposure to waste anesthetic gases has the potential to cause changes in human genome which may lead to increased morbidity.

Key Words: Genotoxicity – Operating room personnel – Waste anesthetic gases.

Introduction

WASTE anesthetic gases are small amounts of volatile anesthetic gases that leak from the patient’s anesthetic breathing circuit into the air of operating rooms during delivery of anesthesia. These gases may also be exhaled by patients recovering from anesthesia. Waste anesthetic gases include both nitrous oxide and halogenated anesthetics such as halothane, enflurane, isoflurane, desflurane, sevoflurane, and methoxyflurane. The halogenated anesthetics are often administered in combination with nitrous oxide. Nitrous oxide and some of the halogenated anesthetics may pose a hazard to hospital workers [1].

There is a great concern that the operating room personnel might be exposed to health risks due to exposure to anesthetic gases. However, whether chronic exposure to these gases is hazardous to the health of anesthetic room personnel is still controversial [2].

Exposure to high concentrations of waste anesthetic gases—even for a short time—may cause the following health effects: Headache, irritability, fatigue, nausea, drowsiness, difficulties with judgment and coordination [1].

Although some studies report no adverse health effects from long-term exposure to low concentrations of waste anesthetic gases, several studies have linked such exposure to genetic damage and cancer among exposed workers. Studies have also reported miscarriages in the spouses of exposed workers and birth defects in their offspring. This reproductive and carcinogenic action in exposed operating room personnel may be related to genetic toxicity of waste anesthetic gases [3].

A meta-analysis suggested that chronic exposure to trace concentrations of anesthetic gases might cause genetic damage [4]. Some studies reported an association between occupational exposure to waste anesthetic gases and an increase in sister chromatid exchanges (SCEs) in lymphocytes for staff working in unscavenged operating room [8]. Other studies did not support the association be-
between occupational exposure to waste anesthetic gases and an increase in SCEs in operating room personnel [6].

Hence, the adverse health effects caused by anesthetic gases in human are of special concern. Among these are the genotoxic effects, including cancer and reproductive toxicity, so genetic biomonitoring of population exposed to potential carcinogens is an early warning system for genetic disease or cancer. It also facilitates identification of risk factor at a time when control measures could still be implemented. Human biomonitoring can be done using different genetic markers. Biomarkers such as chromosomal aberrations, micronucleus test, comet assay and sister chromatid exchange are among the most extensively used markers of genotoxic effects in epidemiologic studies [7,8].

The results of studies of chromosomal damage in operating room personnel were contradictory while findings on sister chromatid exchange were inconsistent. SCE analysis in peripheral blood lymphocytes is a well established technique aimed at evaluating human exposure to toxic agents. Its sensitivity and reliability have made SCE analysis one of the most popular methods in toxicology and human biomonitoring [6].

SCEs are interchanges between DNA replication products at apparently homologous loci. Although the precise molecular mechanisms underlying SCE formation are not fully understood, it has been suggested that they reflect either DNA damage or DNA repair or both [2].

Aim of the work:

This study was carried out to estimate the genotoxic risk of occupational exposure to anesthetic gases in a group of operating room nurses who were examined by conventional cytogenetic methods: Chromosomal aberrations analysis and SCE analysis, and to investigate the possible relation of these findings with age and duration of exposure.

Subjects and Methods

Over a 3 months period starting at April 2009 till June 2009, a cross sectional study was conducted at Kasr El-Aini Hospital.

The study involved 45 subjects were classified into 2 groups. The first group consisted of 27 nurses exposed to waste anesthetic gases in the operating room. The exposed nurses were exclusively females. They worked 8/h/day for 6 days/week. The mean duration of their employment in the operation theatre was 15 years (range 2-31 years) and their mean age was 33.7 years (range 20-50 years).

All the operating rooms had no active waste anesthetic gas scavenging system. The most commonly used anesthetics were nitrous oxide, isoflurane, sevoflurane and desflurane.

The control group composed of 17 nurses who were selected randomly from the same hospital with no history of occupational exposure to anesthetic agents. Both groups were matched for sex, age and socioeconomic status. It was assured that the operating room personnel and the controls did not statistically differ from each other except for occupational exposure.

All examined nurses were non-smokers, working in Kasr El-Aini Hospital.

The studied population were subjected to the following:

- Full history taking, including standard demographic data (age, marital status, etc.) as well as history of medical exposure to X-ray, vaccination or medications, occupational history (working hours/day, years of exposure, use of personnel protective measures, ventilation the workplace, etc...).

Informed consent was obtained from each nurse before the beginning of the study.

- Structural and numerical chromosomal aberrations in peripheral blood lymphocytes using the G-banding technique after chromosomal pretreatment with trypsin followed by staining with dilute Giemsa stain.

Collection of blood samples:

Venous blood sample (1ml) was collected once from all the exposed and control group subjects using heparinized syringes. Blood samples were coded to avoid possible bias. The samples were transported to the laboratory and were processed within 2h after collection.

Chromosomal aberrations (CA) and SCE assay in (peripheral blood lymphocytes):

The CA analysis was conducted following a standard protocol with slight modifications. 0.5 heparinized whole blood was cultured in RPMI with L-GLUTAMINE medium supplemented with 20% fetal bovine serum (FBS), 200ul phyto-haemaglutinin, 100ul penicillin and streptomycin, 100ul antimycotic and 25ul preserved heparin. Each
culture was incubated in 5% CO₂ incubator at 37 °C for 72 hours. Metaphases were obtained by adding colcemide to the cultures at a final concentration 0.4ug/ml 2 hours before harvesting. The cells were collected by centrifugation, re-suspended in a pre-warmed hypotonic solution (0.075M KCl) for 30 min at 37 °C and fixed in acetic acid-methanol (1:3v/v). Chromosome preparations were stained using 4% Giemsa stain. The slides were analyzed using the high power of the light microscope and 25 metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid gap, chromatid deletions, chromatid rings, dicentrics, centromere separation and endomitosis [9].

SCE assay was analyzed as follow: Bromodeoxyuridine (Sigma) was added to a final concentration of 10 µg/ml at the start of the cultures for SCE analysis. The cultures were harvested after 72h cultivation. Harvesting was done as CA but with avoiding excessive light. Slides were stained using 50µg/ml hoechest dye, UV and 4% Giemsa stain then analyzed with high power of light microscope. 25 metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for SCE [10].

Statistical analysis:

Data were checked, coded, entered and analyzed using computer based statistical package for social sciences (SPSS) for windows 7.5 program.

Comparison between quantitative data of the study groups was done using student’s t-test, while comparison between qualitative data was done by chi-square test. Pearson correlation coefficient was used for testing the association between two continuous variables. The “p” value of 0.05 was considered the limit below which the difference of the values would be statistically significant [11].

Results

The results of occupational exposure to waste anesthetic gases on the levels of genetic damage were assessed by CA and SCE analysis. Table (1) represents the distribution of subjects with respect to age and years of employment. The two groups studied had similar demographic characteristics.

Table (2) shows that the prevalence of headache, drowsiness, irritability, fatigue and syncopal attack were reported by 66.6%, 62.9%, 48.1%, 25.9% and 22.2% respectively of the exposed nurses versus 11.1%, 16.6%, 11.1%, 5.5% and 11.1% respectively of the control group. The differences between both group were statistically significance (p<0.05).

Table (3) shows the frequencies of CA (gap, break, deletion, centromere separation, endomitosis and total chromosomal aberrations). In the operating room nurses, a significant increase in total CA indicating chromosomal damage was observed when compared with controls (4.3±3.3 versus 2.3±1.4; p<0.05). The exposed nurses had significantly greater mean for chromatid gaps (3±2.7 versus 1.5±0.9), chromosome deletion (0.51±0.80 versus 0.01±0.02) and endomitosis (18.5% versus 0%) in comparison with controls (p<0.05). As regards centromere separation and chromatid breaks, there was an increase in their frequencies in exposed nurses in comparison with the control group but the increase was not statistically significant (1.78±2.4 versus 0.75±1.2; p>0.05).

Table (4) shows a positive correlation between age and frequency of chromosomal aberrations (chromatid break, deletion, centromere separation and total chromosomal aberrations) (r=0.04, 0.14 0.34, 0.13) but does not reach the significant level (p>0.05). On the contrary, a negative correlation was found between age and chromatid gaps frequency; r=−0.08. As regards SCE, there was a statistically significant positive correlation between age and frequency of SCE in the exposed nurses; r=0.48 (p<0.05). Also, this table shows that years of exposure were positively correlated with CA frequency (chromatid break, deletion, centromere separation and total chromosomal aberrations) (r=0.02, 0.05, 0.39 and 0.11 respectively) but did not significant (p>0.05). On the contrary, years of exposure seemed to have no positive correlation with chromatid gaps frequency when compared by Pearson correlation; r=−0.12. As regards SCE, there was a statistically significant positive correlation between years of exposure and frequency of SCE in the exposed nurses; r=0.39 (p<0.05).

Table (1): Descriptive characteristics of the studied exposed and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed (n=27)</th>
<th>Control (n=18)</th>
<th>t-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20-50</td>
<td>28-53</td>
<td>1.6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mean</td>
<td>33.7</td>
<td>37.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>7</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of work (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2-31</td>
<td>6-35</td>
<td>0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mean</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>6.7</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Frequency of clinical manifestations among the studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed group</th>
<th>Control group</th>
<th>X2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Headache</td>
<td>18</td>
<td>66.6</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>17</td>
<td>62.9</td>
<td>3</td>
<td>16.6</td>
</tr>
<tr>
<td>Fatigue</td>
<td>7</td>
<td>25.9</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Syncopal attack</td>
<td>6</td>
<td>22.2</td>
<td>1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* Statistically significant.

Table (3): Structural chromosomal aberrations and SCE among exposed and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed group (n = 27) (mean ± SD)</th>
<th>Control group (n = 18) (mean ± SD)</th>
<th>Test of sig. t-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chromosomal aberrations</td>
<td>4.3±3.3</td>
<td>2.3±1.4</td>
<td>−2.4</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Breaks</td>
<td>2.1±2.2</td>
<td>1.8±1.1</td>
<td>−.45</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gaps</td>
<td>3±2.7</td>
<td>1.5±0.9</td>
<td>−2.19</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Centromere separation</td>
<td>0.81±1.46</td>
<td>0.55±0.85</td>
<td>0.67</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Deletion</td>
<td>0.51±0.80</td>
<td>0.01±0.02</td>
<td>2.7</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Endomitosis</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>18.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SCE (mean ± SD)</td>
<td>1.78±2.4</td>
<td>0.75±1.2</td>
<td>1.6</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Statistically significant.

Table (4): Pearson’s correlation between (chromosomal aberrations and SCE) and (age, duration of exposure) among the exposed group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>Duration of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Breaks</td>
<td>0.04</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gaps</td>
<td>−0.09</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Deletion</td>
<td>0.14</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Centromere separation</td>
<td>0.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total chromosomal aberrations</td>
<td>0.13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>SCE</td>
<td>0.48</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

* Statistically significant.

Fig. (1): Chromatid break in one of exposed groups (arrow (b) points to the position of break).

Fig. (2): Sister chromatid exchange in one of exposed subjects (arrows pointed to the position of the exchange).
Discussion

The possibility of a potential mutagenic or carcinogenic action of chronic exposure to low concentrations of inhalational anesthetics has been previously studied, with conflicting results.

Workers exposed to excessive amounts of anesthetic gases complain about feeling as if they themselves are anesthetized. They experience drowsiness, irritability, depression, headache, nausea, fatigue and impaired judgment [12].

These behavioral changes are of great concern, particularly in the operating room, where they can compromise surgical success and the health of the operating-room personnel. Assessing the long-term effects of exposure to anesthetic agents is more difficult. The chronic effects of anesthetic gas exposures are usually identified through retrospective epidemiological studies, followed by conformational animal studies. The conclusions that could be drawn in some studies of chronic low-level exposures have been limited due to the lack of quantitative exposure data and heavy reliance on information from questionnaires [13].

However, chronic exposure to waste anesthetic gases has been associated with increased risk of spontaneous abortion in exposed women workers and the wives of exposed men. Other adverse reproductive effects among exposed females include involuntary infertility and infants with low birth weights and with congenital abnormalities [3].

In the present study, only operating room nurses have been chosen as exposed subjects for this work, because they have the most exposure to waste anesthetic gases emanating from the apparatus as they spend more time than other persons working in the operating room (e.g. anesthetists, surgeons, etc.). If ventilators are not used, the level of anesthetic gases in the operating room is 76% higher near the apparatus than elsewhere [14].

Female nurses were only selected in the present study as many researchers concluded that there is a higher sensitivity to hazards of anesthetic gases in woman. Rozgaj, et al. reported that there was a significant increased relative risk values for chromosomal aberrations and micronucleus for woman [8]. Also, Bonassi, et al. confirmed that there was a genetic damage due to exposure to inhaled anesthetics which was significant in woman and not in men [15].

We excluded smoker subjects from our study as several researches demonstrated that smoking had a significant effect on DNA damage as smoking anesthesia persons and smoking control persons presented increased rates of DNA damage [7,16]. Bilban, et al. found that smoking index correlated significantly with the frequency of chromosomal aberrations [8]. This supports the importance of minimizing the risk of unwanted habitual variability (smoking habit), as in our study.

Our results shows that 66.6% of the exposed group reported headache Vs. 11.1% among the control group, with a statistically significant difference (p<0.05). Also, 62.9% of the exposed group had drowsiness Vs. 16.6% in the control group with a statistically significant difference (p<0.05). A statistically significant difference (p<0.05) was also detected between both groups as regards other neurological manifestations (irritability, syncopal attack).

This is in agreement with Zacny, et al. who found in their study that long term exposure to inhalation anesthetic agents may cause headache, depression, anxiety, loss of appetite, loss of memory and also changes in intellectual function [17].

In vitro experiments corroborated those results as Ozer, et al. concluded that behavioral effects were observed from chronic exposure to subanesthetic concentrations of sevoflurane and desflurane in rats [18].

Nitrous oxide exposure was shown to be associated with impaired neurobehavioral performance [19]. Even lower levels of exposure to anesthetic gases can cause an impairment of neurobehavioral performance [20].

In the current study, there were three female nurses out of 27 exposed nurses who were found to have offspring with congenital anomalies. One of the exposed subjects had a pituitary tumor and she was on treatment. Four of them had a past history of abortion.

In accordance to our findings, several researchers reported reduced fertility, increased risk of spontaneous abortion and the development of congenital abnormalities in offspring of operating room personnel exposed to waste anesthetic gases [3,21].

The results of studies of possible genotoxic effects of anesthetics on occupationally exposed subjects are not always comparable. Rozgaj, et al. reported that the increase in sister chromatid exchange frequency was not significant while chromosome aberrations and micronucleus frequency
increased significantly in personnel exposed to anesthetic gases [8].

Also, Chandrasekhar, et al. reported a statistically significant increase in DNA damage as shown by chromosome aberrations, micronucleus frequency and the comet assay in operating room personnel exposed to anesthetic gases [7].

In this study, we found that the incidence of most of the chromosomal aberrations in nurses exposed to waste anesthetic gases was significantly more frequent than that of unexposed nurses in the same hospital.

Our findings showed that the incidence of SCE in nurses exposed to waste anesthetic gases was only slightly higher than in controls (1.78 ± 2.4 in exposed nurses versus 0.75 ± 1.2 in controls) and this increase was not significant. Several researches support our finding including those of Lamberti, et al., Bigatti, et al. and Husum and Wulf while Sardas, et al. and Karelova, et al. reported a significant increase in SCE frequency in medical workers exposed to volatile anesthetics. Natarajan and Santhiya found an increase in SCEs in operating room personnel exposed to anesthetics, although it was not significant [22-27].

In agreement with our results, Hoerauf, et al. found that exposure to even small concentrations of waste anesthetic gases may result in an increased frequency of SCE in operating room personnel. They concluded that this genetic damage is also comparable with smoking 11-20 cigarettes per day [28].

Recently, Bilban, et al. in their study reported that exposure to anesthetic gases induced significant changes in human chromosome as detected by structural chromosomal aberrations, frequency of SCE and micronucleus of anesthetists and other personnel handling anesthetic gases [5].

Also, Wro’nska-Nofer, et al. concluded that occupational exposure to nitrous oxide is associated with increased DNA damage in female nurses exposed to anesthetics [29].

Contrary to what was expected, Pasquini, et al. found in their study a lower frequency of SCE in male anesthesiologists than in controls but micronucleus frequency was significantly higher in female, but not male, anesthesiologists than in controls. Micronucleus analysis seems to be a sensitive index of possible genotoxic effects of occupational exposure to anesthetics, and women appear to be more susceptible to these effects than men [30].

Bozkurt, et al. in their study does not support the existence of an association between occupational exposure to waste anesthetic gases and an increase in SCEs in lymphocytes. This can be explained by the nature of their anesthesia practice which suggests that exposure was likely to be low. It should be noted that some anesthetic gases produce lesions that can be efficiently repaired in mitogen- stimulated lymphocytes in vitro but not in circulating lymphocytes [6].

The mechanism by which the anesthetics induce DNA damage is still unclear. When isoflurane reacts directly with DNA, the most feasible alkali-labile modifications may be alkylation at the N-7 position of purines. Another explanation could be that, anesthetic gases undergo a residual metabolic oxidation or reduction giving rise to reactive products. Radical mediated reactions may also be involved in DNA damage induction [31].

Nitrous oxide may interfere with DNA synthesis by irreversibly oxidizing the cobalt atom of vitamin B12 and reducing methionine and thymidylate synthetase activity [32].

In the operating room nurses of the present study, age and duration of exposure positively correlated with genetic damage as presented by frequency of chromosomal aberrations and SCE. This positive correlation was not significant in case of chromosomal aberrations while it was significant in SCE. Similarly, a cytogenetic investigation of operating room personnel found a positive correlation between chromosomal aberrations and years of employment [5].

On the contrary, a study on operating room personnel using micronucleus test showed that age and duration of employment did not correlate with micronucleus frequency [33]. Also, age and duration of exposure didn’t have a significant effect on DNA damage in a study conducted by Chandrasekhar, et al. [7].

Conclusion:

Results of this study indicated that occupational exposure to a mixture of anesthetic agents induced an increase in the level of genotoxicity which was significant in the frequency of chromosomal aberrations but not significant in SCE.

Besides the genotoxic damage seen in the operating room personnel, some other effects of exposure to waste anesthetic gases were reported such as nausea, dizziness, headache, fatigue and irritability, as well as miscarriages and cancer among operating room nurses and congenital ab-
normalities in their offspring. This outcome is associated with our poorly equipped operating rooms (not having a central high-flow scavenging system and low leakage anesthesia machines, and not having facilities to use low-flow and closed-circuit anesthesia).

We conclude that exposure to even low concentrations of waste anesthetic gases may result in an increased risk of genetic damage which may lead to increased morbidity.

A limitation of our study, as of all other studies on this topic, is that it is not clear whether the observed genotoxic effect was due to the exposure to nitrous oxide, volatile anesthetics, or a mixture of both. Further studies should be performed in personnel solely exposed to nitrous oxide or (single) volatile anesthetics.

The outcome of our study indicates the risk of exposure to waste anesthetic agents in the hospital under study suggesting that anesthesiology practices should be designed to further decrease environmental concentrations of anesthetic gases. The waste anesthetic gas scavenger and air conditioning equipment should be checked frequently and adequate ventilation should be provided. Further, preventive medical examination of all exposed personnel should be carried out periodically, including genetic biomonitoring.

Acknowledgment: The authors are grateful to all nurses involved in this study for their cooperation.

References

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