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Chromosomal Aberrations in Operating Room Nurses Exposed to Waste 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, genotoxic, teratogenic and carcinogenic.

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 (SCE) in peripheral blood lymphocytes.

Results: A significant increase in chromosomal damage in exposed nurses as detected by total chromosomal aberrations, gaps, deletion and endomitosis was detected 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 though 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 including chromosomal aberrations and SCE.

Key Words: *Genotoxicity – Operating room personnel – Waste anesthetic gases – Chromosomal aberrations – Sister chromatid exchange (SCE).*

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 **[1]**.

There is a great concern that patients, physicians, and the operating room personnel might be exposed to health risks due to exposure to anesthetic gases. However, whether chronic exposure to waste anesthetic 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 headache, irritability, fatigue, nausea, drowsiness, and impairment in 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 miscarriages, genetic damage and cancer among operating-room workers. Studies have also reported miscarriages in the spouses of exposed workers and birth defects in their offsprings. This reproductive and carcinogenic action in exposed operating room personnel may be related to genetic toxicity of inhalation anesthetics **[3]**.

A meta-analysis suggested that chronic exposure to trace concentrations of anesthetic gases might cause mutations in DNA **[4]**. Some studies reported an association between occupational exposure to waste anesthetic gases and an increase in sister chromatid exchanges (SC-

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Es) in lymphocytes for staff working in unscavenged operating room (OR) [5]. While others did not support such association [6].

Hence, the undesirable health effects caused by anesthetic gases in human are of special concern. Among these are the genotoxic effects, including cancer and several other genetic diseases. Genetic biomonitoring of population exposed to potential carcinogens is an early warning system for genetic diseases or cancer. It also allows identification of risk factor at a time when control measures could still be implemented. Human biomonitoring can be performed 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 molecular epidemiologic studies [7,8].

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, DNA repair or both [2].

Aim of the work:

The present study was carried out to estimate the genotoxic risk of occupational exposure to anesthetic gases in a group of operating room nurses and to investigate the possible relation of these findings with age and duration of exposure.

SUBJECTS AND METHODS

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

The study involved 45 subjects 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, with an age ranged from 20-50 years and a median of 32 years. They work 8 hours/day for 6 days/week. The median duration of their employment in the operating theatre was 14 years (range 2-31 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 consisted of 18 females nurses who were selected randomly from the same hospital with no history of occupational exposure to anesthetic agents, with an age ranged from 28-53 years and a median of 38 years. The median duration of their employment in the operating theatre was 25 years (range 6-35 years). The operating room personnel and the controls did not statistically differ from each other except for occupational exposure.

All examined nurses were non-smokers.

The studied groups 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 status of the workplace).
- The study was approved by the local Ethics Committee on human research. 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 and determination of Sister chromatid exchange.

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

Venous blood sample (3ml) 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.

The CA analysis was conducted following a standard protocol with slight modifications. Half ml heparinized whole blood was cultured in RPMI with L-glutamine medium supplemented with 20% fetal bovine serum (FBS) (Eurolone, Europe), 200ul phytohaemaglutinin, 100ul penicillin and streptomycin, 100ul antimycotic

and 25ul preserved heparin. Each culture was incubated in 5% CO_2 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 (0.075 M KCI) for 30min at 37°C and fixed in acetic acid-methanol (1:3 v/v). Chromosome preparations were stained using 4% Giemsa stain. The slides were analyzed using the high power of the light microscope and 25 metaphases 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, centomere separation and endomitosis **[9]**.

SCE assay was analyzed as follow: Bromodeoxyuridine (Sigma) was added to a final concentration of 10μg/ml at the start of the cultures for SCE analysis. The cultures were harvested after 72 hours. Harvesting was done as CA but with avoiding excessive light. Slides were stained using 50ug/ml hoechest dye and 4% Giemsa stain then analyzed with high power of light microscope. Twenty five metaphases were screened per each individual. Cells with 46 chromosomes were scored for SCE **[10]**. Examples of the SCE and chromosomal abnormalities are presented Figs. (1-3).

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.

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.

Table (1) shows that the prevalence of headache, drowsiness, irritability, fatigue and syncopal attack were more frequently experienced by the exposed group as compared to the control group. The differences were found to be statistically significant ($p<0.05$).

Table (2) 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, chromosomal deletion and endomitosis were observed 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 not reaching the significant level ($p>0.05$).

As regards the frequencies of SCE, they were slightly higher in the exposed nurses as compared to the control group but the difference was not statistically significant ($p>0.05$).

No correlation was encountered between age or duration of exposure on one side and chromatid breaks, gaps, deletion, centromere separation or total chromosomal aberrations on the other side. On the other hand, a fair positive correlation was encountered between age and duration of exposure on one side and SCE on the other side ($r=0.48$ & 0.39 respectively and $p<0.05$).

Table (1): Frequency of clinical manifestations among nurses exposed to waste anesthetic gases.

Parameters	Exposed group		Control group		*p*
	No.	%	No.	%	
Headache	18	66.6	2	11.1	<0.05*
Drowsiness	17	62.9	3	16.6	<0.05*
Fatigue	7	25.9	2	11.1	<0.05*
Syncopal attack	6	22.2	1	5.5	<0.05*
Irritability	13	48.1	2	11.1	<0.05*

*S = Significant.

Table (2): Structural chromosomal aberrations among nurses exposed to waste anesthetic gases.

Parameters	Exposed group		Control group		*p*
	Range	(Mean ± SD)	Range	(Mean ± SD)	
Total chromosomal Aberrations	1-9	4.3±3.3	1-9	2.3±1.4	<0.05*
Breaks	0-2	2.1±2.2	0-1	1.8±1.1	>0.05
Gaps	0-9	3±2.7	2-4	1.5±0.9	<0.05*
Centromere separation	0-5	0.81±1.46	0-2	0.55±0.85	>0.05
Deletion	0-2	0.51±0.80	0	0.01±0.02	<0.05*
Endomitosis	No.	%	No.	%	<0.05
	5	18.5	0	0	

*S = Significant.

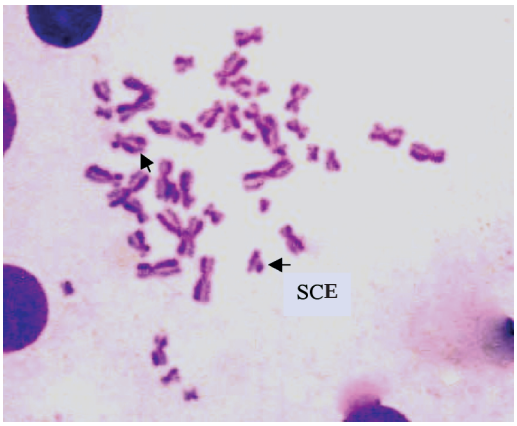

Fig. (1): Hoechst-Giemsa preparation from peripheral blood lymphocytes of a nurse exposed to waste anesthetic gas. The arrow points to sites of Sister Chromatid exchange.

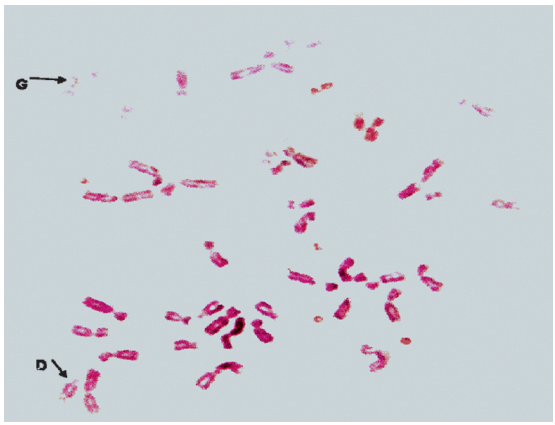

Fig. (2): Giemsa stained chromosomal preparation of a nurse exposed to waste anesthetic gas. Arrow G refers to gap while arrow D refers to deletion.

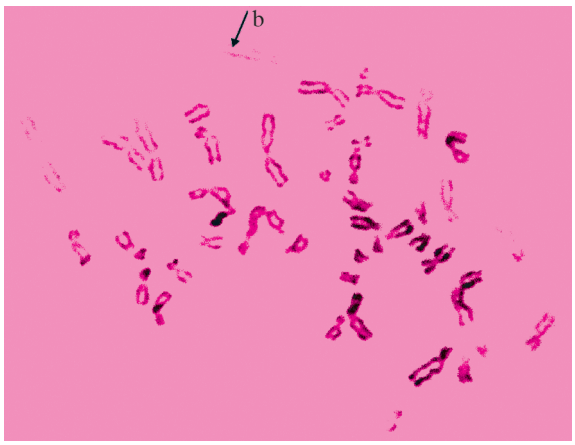

Fig. (3): Giemsa stained chromosomal preparation of a nurse exposed to waste anesthetic gas. The arrow points to chromosomal break.

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 **[6]**.

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 modifications 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 confirmational animal studies. The conclusions that could be drawn in some studies of chronic low-level exposures have been limited due to 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 women. Rozgaj et al. (2001) reported that there was a significantly increased relative risk values for chromosomal aberrations and micronucleus for women **[8]**. Also, Bonassi et al. (1995) confirmed that there was a genetic damage due to exposure to inhaled anesthetics which was significant in women 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 both smoking persons exposed to anesthesia and smoking control persons presented increased rates of DNA damage **[7,16]**. The smoking index correlated significantly with the frequency of chromosomal aberrations **[5]**. This supports the importance of minimizing the risk of unwanted habitual variability (smoking habit), as in our study.

Our results showed that the exposed group reported high frequency of headache, drowsiness and other neurological manifestations (irritability & syncopal attack); the difference was statistically significant ($p<0.05$).

This is in agreement with Zacny et al. (1996) who reported 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. (2006) observed that chronic exposure to sub-anesthetic concentrations of sevoflurane and desflurane is associated with behavioral changes 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 with our findings, several researchers reported reduced fertility, increased risk of spontaneous abortion and the development of congenital abnormalities in the offsprings 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 controversial. Rozgaj et al. (2001) 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. (2006) 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 most of the chromosomal aberrations were significantly more frequent in nurses exposed to waste anesthetic gases than in the unexposed nurses of the same hospital.

Our findings show that the frequency of SCE was only slightly higher in nurses exposed to waste anesthetic gases than in controls, this increase was insignificant. Similar results were obtained by many workers **[5,22-24,28]**. While others reported a significant increase in SCE frequency in medical workers exposed to volatile anesthetics **[6,25-27]**.

Wroska-Nofer et al. (2009) reported that occupational exposure to nitrous oxide is asso-

ciated with increased DNA damage in female nurses exposed to anesthetics **[29]**.

Contrary to what was expected, Pasquini et al. (2001) 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 **[30]**.

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 B_{12} 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 SCE. Similarly, 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]** or DNA damage **[7]**.

In conclusion our study showed that exposure to 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 is attributable 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 danger of exposure to waste anesthetic agents in the hospital, 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).

Our study suggest that anesthesia practices should be designed to further minimize environmental concentrations of anesthetic gases. The waste anesthetic gas scavenger and air conditioning equipment should be included in the operating theater and sufficient ventilation should be provided. Further, preventive health examination of all exposed personnel should be carried out periodically, including genetic biomonitoring.

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