Journal of Microscopy and Ultrastructure 1 (2013) 89–95

Journal of Microscopy and Ultrastructure

journal homepage: www.elsevier.com/locate/jmau

Original Article

Effects of wasted anesthetic gases on human lymphocytes – A genetic study

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ARTICLE INFO

Article history:
Received 3 December 2013
Accepted 19 December 2013

Keywords:
Wasted anesthetic gases
Human lymphocytes
Genetic study

ABSTRACT

Information on potential genetic damage in humans after exposure to waste anaesthetic gases in Egyptian hospitals is scarce. To evaluate the possible genotoxic effects of waste anaesthetic gases, the chromosomal aberrations [CA] and the sister chromatid exchange [SCE] tests, were studied in peripheral blood lymphocytes in 26 operating room personnel (exposed group) currently employed at Tanta University hospitals, in comparison to a group of 13 non-exposed persons (control group), matched by age, sex and smoking habits. The results showed a statistically significant increase in chromosomal aberrations and sister chromatid exchange in the exposed persons in comparison to controls. Also it suggests that exposure to waste anaesthetic gases has the potential to cause changes in human genome.

1. Introduction

During surgery under general anesthesia there is inevitable pollution of the theater by vapors of wasted anesthesitic gases. The degree of pollution depends upon many factors including the quality of the anesthesia machine, the scavenging system, techniques of anesthesia used by anesthesiologist (e.g. open, semi-closed, closed or low flow methods) and lastly the whole ventilation system. It is found that the highest concentration around the anesthesia machine is maximum at the area for anesthesia staff then less at the area for surgeons and scrub nurses [1–4].

Toxicity from exposure to even low levels of wasted anesthesia gases proved to be toxic. Attention to the hazardous effects of theater pollution began in the middle of 1960, with many studies attributed on it. Chronic regular exposure to even low levels of wasted anesthetic gases was found to have critical effects on DNA [5–10].

The stimulus to conduct our study came from the circumstances in our hospital (Tanta University Hospital) as we still use halothane as inhalational anesthetic in many operations due to low cost and at the same time, lack of scavenging systems in our theaters. We think that it is difficult to find such circumstances to conduct this study in other places at this time.

The effects of human exposure to genotoxic mediators can be examined using different genetic indicators. The relevance of chromosomal aberrations (CA) as a biomarker has been highlighted by epidemiological studies suggesting that a high frequency of CA is predictive of an increased risk of cancer [11–17]. In addition, CA is one of the most sensitive cytogenetic parameters [18,19].

Sister-chromatid exchange (SCE) reflects an interchange of DNA sequences between helices in a replicating chromosome [20]. The analysis of SCE has been considered to be a highly sensitive tool to measure mutagenic/carcinogenic potential of various environmental agents [21,22].

So we used the CA and SCE to estimate the genotoxic risk of exposure to halothane on anesthetic personnel.

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2213-879X
http://dx.doi.org/10.1016/j.jmau.2013.12.002

2. Materials and methods

2.1. Subjects

The study involved 39 subjects divided into exposed and control groups. The exposed group was composed of 26 subjects from anesthetic personnel [anesthesia staff, technicians and operating room nurses]. The control group was selected as non-smokers from other non-surgical departments of the hospital. It was composed of 13 subjects matched for age and sex. Each person was interviewed using a standardized questionnaire about demographic data and data about the average period of exposure in hours per day and the duration in years, drug intake, chronic diseases, X-ray exposure in the last 3 months and smoking.

2.2. Description of the workplace

The operating rooms were in range air volume of 130–200 m^3. Air was conditioned by ordinary air conditioners. No laminar flow system producing an air exchange. The anesthetic gases used were halothane in most cases (78.6%) and isoflurane (22.4%). The exhaust outlets of the anesthesia machines drain to the operating rooms atmosphere [scavenging systems not available].

2.3. Lymphocyte cultures, metaphase preparation and, SCE

Blood (1 ml) was drawn into heparinized vacutainers. Blood samples were coded to avoid possible bias and to be analyzed blindly. Cultures were set up within minutes after collection according to the chromosome analysis protocols described by Gosden (1994) [23]. The protocol for sister-chromatid exchange described by Latt (1981) [20], was used. Freshly obtained blood samples were cultured in [4 ml] RPMI 1640 medium [Bio-West], supplemented with (1 ml) fetal bovine serum [Bio-West], 10% phytohemagglutinin (Bio-Chrome), and 0.1 ml penicillin, streptomycin (Sigma–Aldrich). For each culture, 0.5 ml of whole venous blood was inoculated at 37 °C into a sterile plastic culture tube with 5 ml of complete culture medium, for 72 h.

For SCE analysis and cell kinetics, Bromodioxuridine (BrdU) was added to the culture after 24 h from initiation, at a final concentration of 5 μg/ml. Colcemid was added to all cultures 2 h before harvesting at a final concentration of 0.025 μg/ml.

At the end of the 2 h, tubes were centrifuged at 1000 rpm for 10 min, the supernatant was removed, the cells were mixed thoroughly, and 5 ml of prewarmed hypotonic solution (0.075 M KCl) was added. The cells were then incubated at 37 °C for 20 min. The tubes were centrifuged again at 1000 rpm for 10 min, the supernatant was removed, the pellet was mixed thoroughly, and 5 ml of fresh fixative (one part acetic acid to three parts methanol) was added drop by drop. This fixative procedure was repeated twice more and the tubes were centrifuged for the last time, then the cell pellet was resuspended in a small volume (0.5–1.0 ml) of fresh fixative and dropped onto a clean microscope slide and dried at room temperature. The coded slides were scored blindly under a microscope. For each subject, at least two lymphocyte cultures were set up.

2.4. Cytogenetic analysis

A total of 50 well-spread metaphases (25 from each of at least two replicate slides) with 46 ± 1 chromosomes from different cultures were analyzed per subject. Chromosome- and chromatid-type aberrations, including gaps, were recorded. A chromatid lesion shorter than the diameter of the chromatid was classified as a chromatid gap and a lesion with a length equal to or longer than the diameter of the chromatid was classified as a chromatid break. The chromosomes with one of the arms shorter than its sister were considered having deletions.

The dicentric chromosome was diagnosed in chromosomes with two centromeres. The occurrence of a part of chromosome or a chromosome without centromere, was classified as acentric fragment. The total chromosomal aberrations including and excluding gapes were calculated and correlated to the duration of exposure and the age. The number of metaphases/500 cells for each subject was counted to determine the mitotic index (MI) [24].

For cell cycle kinetics, first mitotic division (M1) where both chromatids of each chromosome stained dark, second mitotic division (M2) where the chromosome appeared with one chromatid stained dark and its sister chromatid stained light (harlequin chromosome), and third mitotic division (M3) where the metaphases have some chromosomes lightly stained and others with harlequin appearance, M1, 2, 3 metaphases were counted from a total of 100 metaphase cells per subject. The replicative index (RI) was calculated as $RI = 1 \times M1 + 2 \times M2 + 3 \times M3/100$ [10]. Fifty-well spread and completely differentiated second division metaphase (M2) plates were counted for each subject to compute the mean SCE frequency/cell.

2.5. Statistical analysis

The Student t-test and Mann–Whitney *U*-test were used for statistical analysis and interpretation of the results regarding the frequencies and types of CA, including and excluding gaps, MI, PRI and SCE. The level of statistical significance was set at $P < 0.05$. The correlation between the duration of exposure and the frequency of CA were admitted using the Pearson correlation coefficient and trends, with the SPSS computer program.

3. Results

The job-related levels of genetic damage in anesthesiologists' staff as a result of exposure to waste anesthetic gases were assessed as regard to CA and SCE analysis. Table 1 represents the distribution of subjects with respect to age, sex, smoking, hours of exposure per day and years of exposure. The two studied groups had nearly matched demographic characteristics. The mean age of the exposed group was 31.192 ± 3.06, ranging from 20 to 58 years, and that of controls was 39.53 ± 10.42, ranged from 21 to 55 years. The

Table 1
characteristics of the studied groups.

Variables	Exposed personnels No. = 26	Controls No. = 13
Age	Mean ± SD 31.192 ± 3.06 Range = 20–58	Mean ± SD 39.53 ± 10.42 Range = 21–55
Sex		
Males	15 (57.7%)	6 (46.2%)
Females	11 (42.3%)	7 (53.8%)
Smoking		
Non-smokers	24 (92.3%)	13 (100%)
Smokers	2 (7.7%)	0 (0%)
Hours of exposure	Mean ± SD 9.077 ± 2.93 Range = 2–12 h/d	Not exposed
Years of exposure	Mean ± SD 10.89 ± 1.93 Range = 1–30	Not exposed

duration of exposure ranged from 1 to 30 years, for an average period of 10 h per day for at least 6 days per week.

3.1. Chromosomal aberration assay

The chromosomal pattern of the studied groups was analyzed for structural chromosomal aberrations (CA). The entity results regarding the frequency and type of CA are shown in Table 2. In the operating room personnel there was a significant increase in the total chromosomal aberrations including and excluding gaps (CAi, CAe), when compared with controls ($P < 0.05$).

The normal human metaphase chromosomes appeared spread to form the classic four arm structure, a pair of sister chromatids attached to each other at the centromere. The shorter arms are called *p* arms and the longer arms are called *q* arms. Human cells have 23 pairs of chromosomes, giving a total of 46 per cell (Fig. 1).

The control group showed four types of the chromosomal aberrations, however, the exposed personnel metaphases showed many types of structural aberrations that appeared singly or in combination inside the same cell (Fig. 2).

All types of CA showed a statistically significant increase in the exposed group with respect to controls except for dicentric chromosomes (Figs. 2 and 3) as the difference was not significant.

Chromatid\chromosome gaps (Fig. 4) were the most frequent CA observed in the both groups, where the mean ± SD of them were (4.923 ± 2.682 and 0.846 ± 0.128) for the exposed and control groups respectively, followed by the second frequent chromosomal aberration which

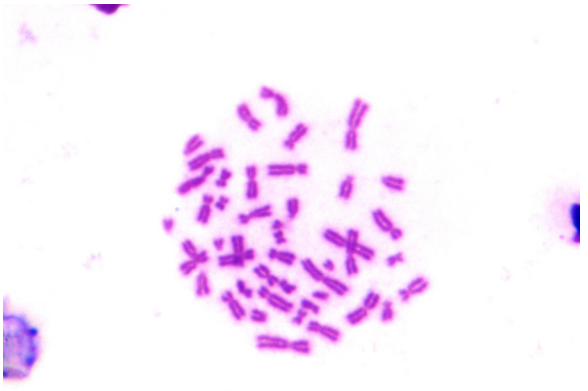

Fig. 1. Photomicrograph of normal human metaphase spreads from phytohemagglutinin-stimulated peripheral blood lymphocytes which appears to form 46 chromosomes with the classic four arm (a pair of sister chromatids attached to each other at the centromere.)

Table 2
Difference between control and exposed groups as regards the studied variables.

Variables	Exposed personnels	Controls	*P*-value
Gap	Mean ± SD 4.923 ± 2.682 Range = 0–9	Mean ± SD 0.846 ± 1.28 Range = 0–3	<0.001**
Break	Mean ± SD 1.269 ± 1.282 Range = 0–5	Mean ± SD 0.769 ± 1.23 Range = 0–4	0.021*
Del	Mean ± SD 1.00 ± 1.1 Range = 0–3	Mean ± SD 0.00 Range = 0	<0.001**
Dicentrics	Mean ± SD 0.730 ± 0.769 Range = 0–3	Mean ± SD 0.769 ± 1.091 Range = 0–4	0.965
Fragment	Mean ± SD 2.846 ± 1.897 Range = 0–7	Mean ± SD 1.461 ± 1.898 Range = 0–6	0.042*
CAi	Mean ± SD 10.692 ± 4.994 Range = 0–18	Mean ± SD 4 ± 4.397 Range = 1–17	<0.001**
MI	Mean ± SD 68.923 ± 30.339 Range = 23–143	Mean ± SD 30.154 ± 22.218 Range = 10–90	<0.001**
CAe	Mean ± SD 5.962 ± 2.863 Range = 0–11	Mean ± SD 3.154 ± 3.46 Range = 1–14	0.020*
SCE	Mean ± SD 4.91 ± 1.97 Range = 0–8	Mean ± SD 3.06 ± 1.59 Range = 1–13	<0.001**
PRI	Mean ± SD 1.77 ± 0.51	Mean ± SD 1.53 ± 0.35	0.865

CAi, chromosomal aberrations including gaps; CAe, chromosomal aberrations excluding gaps; Del, deletion; MI, mitotic index; PRI, proliferative rat index.
** $P < 0.001$.
* $P < 0.05$.

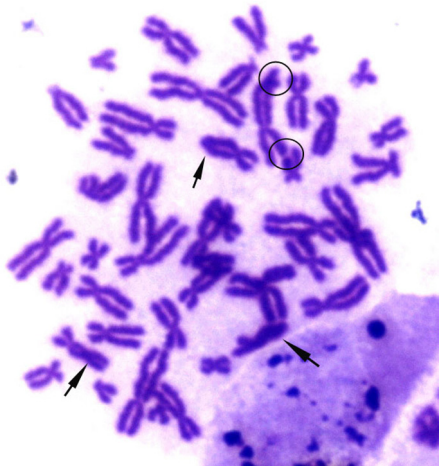

Fig. 2. Photomicrograph of human metaphase spreads showing dicentric chromosomes (arrows), deletions (circles), the upper circle shows dicentric and deletion.

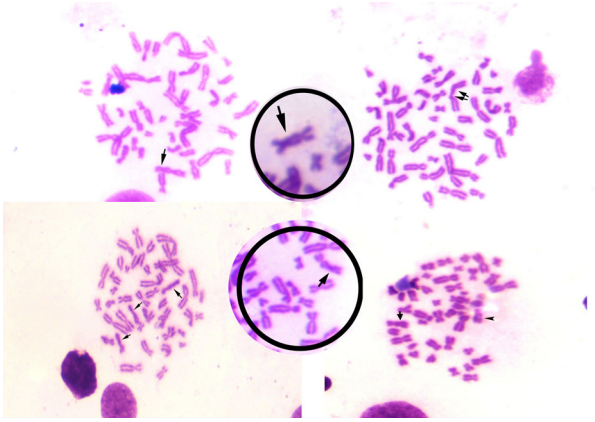

Fig. 3. Photomicrographs of human metaphases spread showing dicentric chromosomes (arrows).

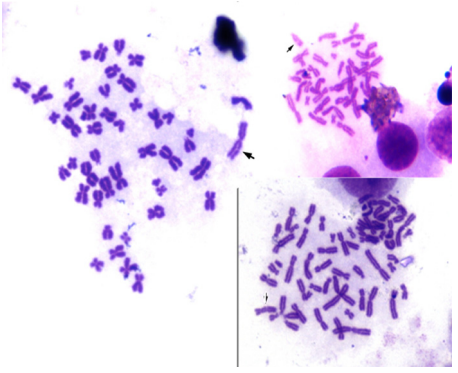

Fig. 4. Photomicrographs of human metaphases spread showing chromatid gaps (arrows) and chromosome gap (right down metaphase).

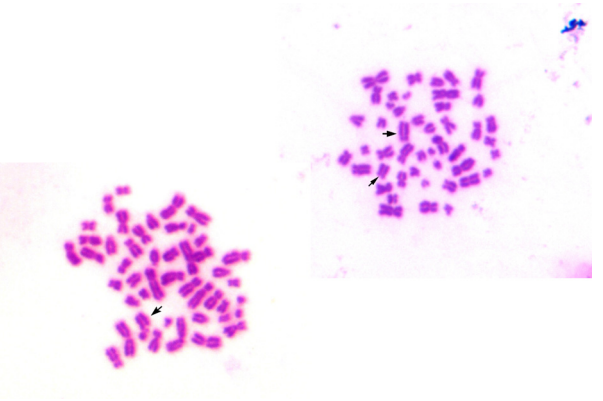

Fig. 5. Photomicrographs of human metaphases spread with 47 chromosomes showing acentric chromosomes (fragments) (arrows).

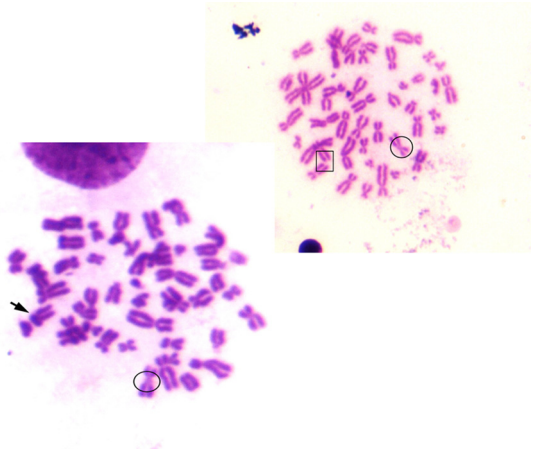

Fig. 6. Photomicrographs of human metaphases spread showing chromosomal breaks (circles), deletions (rectangle) and acentric fragment (arrow).

was the acentric fragments (Figs. 5 and 6) in the mean of (2.846 ± 1.897 and 1.461 ± 1.898).

Chromatid\chromosome breaks (Fig. 6); were also detected to be significantly higher in exposed group in oppose to control one (1.269 ± 1.282 and 0.769 ± 1.230). However deletions (Figs. 2, 6 and 7) were the structural aberrations which are seen only in the examined metaphases of exposed persons where the mean was (1.00 ± 1.10).

Regarding the mitotic activity of the cells, there was a significant increase in the mean of mitotic index of exposed personnel (68.923 ± 30.339) compared to that of the control (30.154 ± 22) (Table 2).

Years of exposure to waste gases of anesthesia showed a positive correlation to total CA frequency but age seemed to have no alliance with CA frequency when compared by Pearson test.

3.2. Sister chromatid exchange

Regarding the sister chromatid exchanges in the cells (Fig. 8), there was a significant increase in the mean of the exchanges between the sister chromatids per cell in

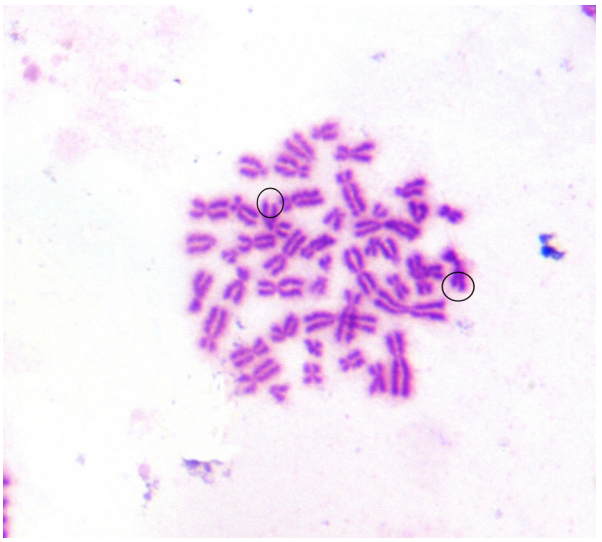

Fig. 7. Photomicrograph of human metaphase spreads showing deletions (cirles).

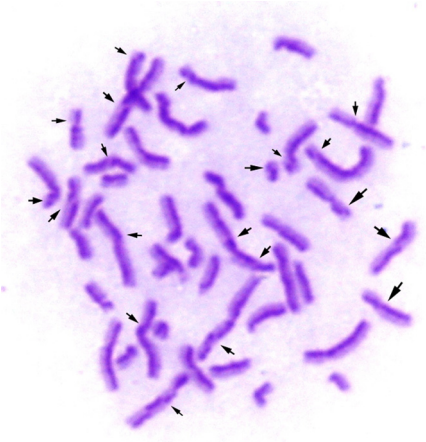

Fig. 8. Photomicrograph of human metaphase spreads showing 20 SCEs (arrows).

exposed personnel (4.91 ± 1.97) compared to that of the control (3.06 ± 1.59), ($P < 0.001^{**}$) (Table 2).

The values for the proliferative index proved non significant difference between exposed and control people (Table 2).

4. Discussion

Hospital persons are occupationally exposed to various agents known or suspected to induce chromosome damage; the focused studied being waste anesthesia gases. Exposure measurements taken in operating rooms during the clinical administration of inhaled anesthetics denote that dissipate gases can escape into the room air from assorted components of the anesthesia release system. In addition, chosen anesthesia techniques and inappropriate practices also can contribute to flee of waste anesthetic gases into the operating room environment.

Over the years there has been significant perfection in the control of anesthetic gas pollution. However, occupational exposure to waste gases still occurs [10].

The aim of this study was to evaluate the genotoxic damage in persons employed as operating room personnel [anesthesia unit doctors, nurses and technicians] in Tanta University hospital, where they are working in theaters with low measures of health safety as well as using halothane which proved to be genotoxic [5,6] and so prohibited in different countries as a type of anesthesia for humans. In addition, many persons of the anesthesia staff suffered from or even died with different types of cancers.

We believe in the fact that specially in cancers, a cause-effect relationship has never been proved, but also chronic exposure to toxicants represents a relevant risk factor for the development of diseases associated with genetic damage. This investigation was conducted to evaluate until which extent this staff is affected, by utilizing the CA and the SCE tests.

Although actual evidence supporting the role of chromosomal alterations in early stages of cancer has been available for a long time, the first epidemiological data showing that the frequency of CA in peripheral lymphocytes may predict cancer incidence in human populations were published in early 1990s [14,25–28]. The black box that remains the core of a cancer cell is now becoming dark gray.

CA are small fractions of a huge amount of changes in chromosomal DNA and reflect an enormous plasticity of the genome which has far reaching consequences for evolution [29].

To understand how the chromosomes are aberrant, gaped or braked, it is hypothesized that breakage in DNA was connected to “replication.” As cells divide, the DNA inside those cells must duplicate, which is called replication. It is revealed that the chromosomes were breaking because replication was stalled. It is found that the fragile sequence actually stops replication, so when replication gets there, it has trouble, it stops, it pauses, it can’t go further very easily. Most of the time, chromosomes break and heal correctly. The problem arises when they do not heal correctly and instead are deleted or rearranged; cancer cells almost always have some sort of deletions or rearrangements [29].

Experimental analyses have shown that DNA double strand breaks [DSB] are the principal lesions in the process of CA formation [30–32]. The majority of chemical mutagens are not able to induce DSB directly but lead to other lesions in chromosomal DNA which during repair, or DNA synthesis, may give rise to DSB and eventually to CA [33,34].

More than two DSB can be involved in the formation of CA by the DSB-repair mechanisms, and it is imaginable that these different mechanisms can participate in the formation of complex aberrations [29].

Sister-chromatid exchange [SCE] is a reciprocal exchange of DNA segments between sister-chromatids at identical loci. This event occurs during DNA synthesis, although the underlying mechanism is not clear up till now, it is known to be associated with DNA replication and repair [20]. The assessment of SCE has been considered to be a highly sensitive tool to evaluate

mutagenic/carcinogenic potential of various environmental agents. This test has gained popularity in order to detect and differentiate among chromosome fragility caused by human diseases that may predispose to neoplasia [21,22].

In the present study, peripheral blood lymphocytes from controls and persons exposed to waste gases of anesthesia examined for the incidence of chromosomal aberrations. The results showed significant increase of total chromosomal aberrations [including and excluding gaps] in exposed personnel than control. These chromosomal aberrations were in the form of [gaps, breaks, fragments, dicentrics, and deletions that not found in controls]. In agreement with our results, Lamberti et al. (1989) [35], who compared hospital workers occupationally exposed to low level anesthetic gases with normal population, and a significant difference were observed for the frequencies of the different chromosomal abnormalities examined, they also added that operating room environments should be closely monitored to minimize risk factors while safeguarding patients. In fact, however mild in the long run, the effects could add up to and prove harm to personnel.

The increased frequency of aberrations in the exposed subjects in this study confirmed previous results of Rozgaj et al. (1999) [36], and the results of other authors [37–41].

Rozgaj et al. (2001) [42] examined whether chromosomal damage could serve to indicate exposure to anesthetics and recorded that, while the increase in sister chromatid exchange frequency was not significant, chromosome aberrations frequency increased significantly.

A significant higher rate of cellular genetic damage in terms of SCEs were recorded in this investigate regarding the exposed opposing the non-exposed persons. These results coincide with that of Bilban et al. (2005) [41] and also with Hoerauf et al. (1999) [7] who studied the incidence of waste anesthetic gases induced sister chromatid exchanges in lymphocytes of operating room personnel and concluded that exposure to even trace concentrations of waste anesthetic gases may cause genetic damage comparable with smoking 11–20 cigarettes per day.

In agreement with our results Chandrasekhar et al. (2006) [10] evaluated the genetic damage in operating room personnel exposed to anesthetic gases and found chromosome aberrations and micronucleus frequencies increased significantly in the study subjects in comparison to the controls.

Similar results for CA were obtained by some authors [35,37,43], even as others [38,44] reported also a significant increase in SCE frequency in medical workers exposed to volatile anesthetics. However, other studies could not identify a significant increase in SCE frequencies in the exposed groups than in controls as Natarajan and Santhiya (1990) [45] who found an increase in SCEs in medical personnel exposed to anesthetics, although it was not significant.

In the same consequence Szyfter et al. (2004) [5] estimated the genotoxic effect of exposure to halogenated anesthetics in 29 operating room personnel but using comet assay and compared with those from a control non-exposed group. No significant differences were detected between the groups.

Unfortunately there were some limitations of the study. We could not use a direct reading instrument to measure the occupational exposure of anesthesiologists to inhaled anesthetics. Therefore it was not possible to draw a concise comparison for dose genetic damage relationship. However we replaced it by duration of exposure to aberration correlation.

In our study there was a positive correlation between the total CA frequency and years of exposure to waste gases of anesthesia. Lamberti et al. (1989) [35], analyzed how frequency and years of employment related: their results highlight a positive correlation in the case of chromosome aberrations both when the entire exposed population was considered and when personnel from the different operating rooms were considered separately.

Eroglu et al. (2006) [46] suggested that two major points should be considered when evaluating the current situation of occupational risks of inhaled anesthetics. First, following the recommendations based on the epidemiological data to reduce health risks by minimizing occupational exposure, the working environment can be improved technically, possibly resulting in small concentrations of waste anesthetic gases; and, second, halothane, which is classified as potentially embryotoxic and genotoxic, can be substituted with isoflurane.

There is no apparent reason for the lack of agreement between reported data obtained in various laboratories. Small variation in laboratory techniques may be a factor, but even when technical aspects are controlled, there are still differences which can be due to scorer efficiency or chosen cells to be analyze. So that a background data is essential for any laboratory trying to study toxicant induced chromosomal aberrations in exposed persons.

Nevertheless, we can conclude that even an exposure for a short duration to waste anesthetic gases leads to an increased risk of genetic damage. Whether the genetic damage established could result in cancer or other unpromising health outcome remains uncertain, provided an otherwise healthy subject has sufficient DNA repair mechanisms, and no other health risks—for example, cigarette smoking, immune deficiencies, consuming illness, or other additional potential genetic hazards are present. Without doubt, these increased CA and sister chromatid exchange rates may be hazardous in the long term and need further investigation.

The outcome of our study indicates the danger of exposure to waste anesthetic agents in the hospitals suggesting the need to further minimize the exposure. The waste anesthetic gas scavengers, laminar air flow and air conditioning equipment should be submit an application and checked frequently and adequate ventilation should be provided. Further, preventive health examination of all exposed personnel should be carried out from time to time.

However about the international altitude this study suggesting the need for international strict roles hold up on evidence based medicine for protection of operating room personnel.

Conflict of interest

No conflict of interest.

Acknowledgements

The authors express their sincere thanks to the volunteers in the hospital departments for providing facilities and encouragement during the study. This work was supported by the funds provided by Tanta University Egypt.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmau.2013.12.002.

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