

# Evaluation of The Genotoxic Effects of Inhalation Anesthetics on the Patients after Single Exposure Using Random Amplification of Polymorphic DNA (RPAD-PCR) Technique

Walaa A. Allam, Soheir A. Mohamed, Asmaa M. Khalaf<sup>1</sup>, Aida A. Mahmoud<sup>2</sup> and Wael A. Mahmoud<sup>3</sup>

<sup>1</sup>Department of Forensic Medicine and Clinical Toxicology.

<sup>2</sup>Department of of Medical Biochemistry.

<sup>3</sup>Department of Anesthesia.

Faculty of Medicine, Sohag University, Sohag, Egypt.

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## Abstract

**Introduction:** DNA is continuously exposed to a variety of biological, chemical and physical agents that may alter its structure and modify its function. Anesthetic gases used in general anesthesia procedures have been claimed to cause genotoxicity.

**Aim of the work:** The present study aimed to evaluate the genotoxic effects of two commonly used anesthetic gases, isoflurane and sevoflurane after single exposure by RAPD PCR method.

**Methodology:** The study was carried out on 50 patients admitted to Sohag University Hospitals for different surgical indications and exposed to inhalation anesthetics for the first time.

**Results:** Obtained results revealed that the exposure to inhalation anesthetics led to DNA changes observed as a difference in the RAPD PCR pattern of the patient before and after exposure to the inhalation anesthetic. Eighty percent of patients exposed to inhalation anesthesia for the first time had a change in the RAPD PCR pattern in the form of band gain, increased band intensity, band loss, or decreased band intensity, however, isoflurane was less mutagenic than sevoflurane .

**Conclusion:** Inhalation anesthetics had genotoxic effect detected by RAPD PCR method.

**Recommendations:** It is recommended to examine patients after a period of exposure to inhalation anesthesia to indicate whether the DNA changes are permanent or temporary. It is important to extend the research using large sample size and different anesthesia protocols.

**Key words** anesthetics, Isoflurane, Sevoflurane, RAPD PCR.

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## Introduction

The inhalation agents used in operations include the fluorinated ethers; isoflurane, sevoflurane, and desflurane and the gas nitrous oxide (N<sub>2</sub>O). 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 (Al-Ashour et al., 2014).

There is a great concern that operating room personnel as well as patients might be at risks from anesthetic gases (El-ebiary et al., 2013). Health hazards connected with an occupational exposure to inhalation anesthetics is not sufficiently documented but some reports on nephrocytotoxicity, hepatotoxicity and carcinogenicity have been published .A chronic exposure to halogenated anesthetics can also affect human reproduction (Deckardt et al., 2007).

Genotoxic effects in particular due to exposure to anesthetics has been the subject of continuing debate (Szyfter et al., 2016). The damage to the genetic material is caused by the interactions of the genotoxic substance with the DNA structure and sequence. These genotoxic substance interact at a specific location or base sequence of the DNA structure causing lesions, breakage, fusion, deletion or non-disjunction leading to damage and mutation. Oxidants as well as free radical when present in the cellular system can adversely affect and alter the structure of lipids, proteins as well as DNA (Sabita et al., 2017).

There are several assays available to study the genetic toxicity of inhalation anesthetics. RAPD PCR technique is a simple, sensitive and reliable method that

utilizes molecular biology knowledge to assess genotoxicity (Zhiyi and Haowen 2004).

The RAPD technique is based on the polymerase chain reaction (PCR). A target DNA sequence is amplified with the help of arbitrary primers, a thermostable DNA polymerase, dideoxy nucleotide triphosphates, magnesium and reaction buffer. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and an elongation step (Atienzar and Jha, 2006).

The aim of the present study was to evaluate the genotoxic effects of two commonly used inhalation anesthetics (isoflurane and sevoflurane) after a single exposure by RAPD method.

## Materials and Methods

A prospective, cohort, controlled study was conducted on 50 patients (30 females & 20 males) admitted to Sohag University Hospitals for different surgical indications and who were exposed to inhalation anesthetics (isoflurane & sevoflurane) for the first time. Their ages ranged from 18 to 50 years.

They were classified according to the period of exposure during the operation. Two blood samples were withdrawn one before the induction of anesthesia and the other after the end of the operation. The department of anesthesia approved the withdrawal of samples from patients to conduct this study under the supervision of anesthesiologists. The study was approved by the Scientific Ethical Committee of Faculty of Medicine, Sohag University. An informed written consent was discussed and taken from all the participants in the study.

### Exclusion criteria

Children and elderly individuals (more than 50 years old), persons taking chemotherapeutic drugs or radiotherapy, Drug abuse or cigarette smoker and those with infectious disease.

Patients included in the present study had strict exclusion criteria to minimize the influence of possible confounding factors. Only non smokers were selected since smoking has been reported to play a causative role in higher incidence of DNA damages. This high risk for genetic changes was also recorded among patients with genetic or malignant disorders, diabetes mellitus as well as radiation exposure (Angelini et al., 2005). Chronic infection also excluded as it generates a milieu of inflammatory cytokines that leads to inflammatory microenvironment, a critical modulator of carcinogenesis. The persistent infection and chronic inflammation changes somatic cells by the influence of associated microbes and epigenetic factors (Fernandes et al., 2015). Treatment with many of chemotherapeutic agents results in the generation of ROS in the cell that can directly induce a wide array of DNA damage including base oxidation, sugar fragmentation and single strand DNA breaks (Derek and John, 2013).

### General anesthesia procedure

Standard clinical monitoring was performed: electrocardiogram, peripheral oxygen saturation (SpO<sub>2</sub>), non-invasive arterial pressure (systolic and diastolic), end-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) and ISF and monitoring of neuromuscular blockade. All patients were premedicated in the operating room with intravenous (IV) benzodiazepine midazolam (0.05mg/kg). Anesthesia was induced with opioid fentanyl (2 µg/kg IV), hypnotic agent propofol (2 mg/kg IV) and rocuronium bromide (0.5mg/kg IV), which is a neuromuscular blocker that was given to facilitate endotracheal intubation. The lungs were mechanically ventilated with 40% oxygen (0.5 l/min) in air (1 l/min). Isoflurane (Abbott) at 1.0 minimum alveolar concentration (1.2%) was administered by inhalation or sevoflurane at 1.0 minimum alveolar concentration (2%) (Braz and Karahalil, 2015). Adequacy of anesthesia during maintenance was assessed by haemodynamic responses, and additional doses of fentanyl (1µg/kg) and rocuronium (0.1 mg/kg) were used when inadequate anesthesia was noticed. The neuromuscular block was reversed with neostigmine (50 µg/kg IV) and atropine (10 µg/kg IV) at the end of surgery (Braz et al, 2011)a .

### Materials

#### 1. Chemicals for DNA extraction

Thermo Scientific GeneJET™ Whole Blood Genomic DNA Purification Mini Kit. It is composed of; red blood cell lysis solution, extraction solution, wash buffers, microspin columns and collection tubes.

#### 2. Chemicals for DNA Amplification

**PCR master mix** is a premixed ready-to-use solution containing bacterially derived Taq DNA polymerase, dNTPs, MgCl<sub>2</sub> and GENETAQ buffer at optimal concentrations for efficient amplification of DNA templates by PCR. It was Stored at -20.

**3. Chemicals for DNA amplification detection:** 50 X TAE Electrophoresis Buffer, agarose (100g), ethidium bromide (10 mg/ml) DNA ladder (50 mg) and DNA loading dye.

**Primers:** Arbitrary ten mer primers as shown below.

Primer	Sequence
Primer 1	5`-CCG GCT ACG G-3`
Primer 2	5`-CAG GCC CTT C-3`
Primer 3	5`-TAC GGA CAC G-3`
Primer 4	5`-AGC TTC AGG G-3`
Primer 5	5`-AGG CAT TCC C-3`
Primer 6	5`-GGT CTG AAC C-3`
Primer 7	5`-TAG GCT CAC G-3`
Primer 8	5`-ACG GTA CAC T-3`
Primer 9	5`-GTC CTC AAC G-3`
Primer 10	5`-CTT CAC CCG A-3`

### Methods of the study

After taking informed consent from the department of anesthesia and the patients, all the participants were subjected to the following

**1-pre-designed questionnaire** was used to collect data from all participants about personal habits as smoking or alcohol, any past medical problems, family

history of any genetic defects and previous exposure to anesthesia.

## 2-DNA study

**Sampling:** two ml of venous blood were collected from each participant; First sample before the start of anesthesia (control) and second sample after the end of the operation. These samples were put in EDTA tubes and kept at 4°C up to one day for DNA extraction .

**DNA extraction** DNA extraction was done using Thermo Scientific GeneJET™ Whole Blood Genomic DNA Purification Mini Kit according to manufacturer' instruction . Extracted DNA concentraion was measured by nanodrop spectrophotometer (Quawell nanodrop spectrophotometer) (Q5000).

**DNA amplification procedure (RAPD PCR)** (Atienzar and Jha, 2006): This was done by PCR technique using 10- mer primers as shown in **table 1**.

To a PCR tube, all the ingredients were added in order: 25 ul master mix, 1ul of each primer (total is 10 ul primers) and adjustd DNA concentration (X ul purified DNA)and distilled water was added up to 50 ul ( Xian et al., 2005).The tube was put on vortex for 1-2 seconds to mix the contents thoroughly.

DNA amplification cycle: carry out using Biometra apparatus using the following reaction conditions; Initial denaturation at 94°C for 5 minutes the 35 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min. Final extension at 72°C for 5 minutes and then cooling at 4°C. Amplified DNA fragments were seperated by agarose gel electrophoresis, stained by ethidium bromide and visualized using gel documentation system (Alpha Metrix Biotech).

## Statistical analysis

Statistical package for social sciences (IBM-SPSS), version 24 (May 2016); IBM- Chicago, USA was used for statistical data analysis.

Data were expressed as mean, standard deviation (SD) or number and percentage (N and %). Student-t test was used to compare the means between two groups, and one-way analysis of variance (ANOVA) test was used to compare means of more than two groups. Chi- square test was used to compare percentages of qualitative variables.

## Results

The majority of cases had operation period between 1-2 hours (80% of cases), with mean operation time 1.86; however, the operation time reached much higher values for a minority of cases, up to six hours as shown in table (1).

There was no significant statistical difference between the mean value of age of cases received isoflurane or those received sevoflurane as shown in table (2).

The type of inhalation anesthetic was compared to the operation period among studied cases. The mean operation period among those received isoflurane (2.064

hours) was significantly longer than those received sevoflurane (1.36 hour) as shown in table (3).

## Results of RAPD PCR analysis

The genotoxic effects of the inhalation anesthetic were observed as; band loss, decreased intensity of the bands, band gain or increased intensity of the bands in the pattern of the RAPD PCR as shown in figure (1) (2).

**Table (4)** shows results after single exposure to anesthetic among studied cases, it reveals that eighty percent of patients exposed to inhalation anesthesia for the first time showed band change, and only 10 cases (20%) showed no change in bands in RAPD PCR pattern. The most common change was combined band gain & increased band intensity or band loss & decreased band intensity in the same patient. Regarding individual changes, the most common change was band gain (48%); followed by band loss (38%); then decreased band intensity (32%) and lastly increased band intensity (16%).

As regard to the comparison between RAPD PCR results after single exposure to inhalation anesthetic and type of anesthetic used among studied cases, Patients received isoflurane (n=39) showed DNA changes in 76.9% (n=30) of cases and no DNA change in 23.1% (n=9) of cases, while those received sevoflurane (n=11) revealed DNA changes in 90.9% (n=10) of cases and no DNA change in 9.1% (n=1) of cases.

The band changes showed significant statistical difference between the patients received isoflurane and those received sevoflurane. The vast majority of those received sevoflurane had combined band loss and gain (81.8%) compared to only 23.1% among those received isoflurane . Also, only one case (9.1%) of the sevoflurane group showed no band change, compared to 23.1% among isoflurane group as shown in table (5).

Regarding the comparison between RAPD PCR results after single exposure to anesthetic and age among studied cases. Although band loss was associated with older age and band gain was associated with younger age. Non-significant statistical difference was detected as shown in table (6).

Regarding the comparison between RAPD PCR results after single exposure to inhalation anesthetic and gender among studied cases, the study revealed non-significant statistical difference between band change according to the patients gender as shown in table (7).

As regard the comparison between RAPD PCR results after single exposure to anesthetic and operation period among studied cases .The present study revealed that band change was significantly related to the operation period; the shorter the operation period, the likely to show "no band change". Also, the maximum mean operation period was seen among those having band loss (2.59 hours) followed by band gain (2.14 hours) then combined band loss and gain (1.56 hours) and lastly no change (1.30 hours) (P value = 0.001).

**Table (1): The duration of operation (in hours) and number of patients.**

Duration of operation (hrs)	1	1.5	2	2.5	3	4	6	Mean $\pm$ SD
N (%) of the patients	13 (26)	11 (22)	16(32)	5(10)	3(6)	1(2)	1(2)	<b>Total=50(100)</b> 1.860 $\pm$ 0.892

*N*: number, *Hrs*: hours, *SD*: standard deviation.

**Table (2): Student t-test showing comparison between type of inhalation anesthetic used and age of the participants.**

Type of drug	Isoflurane	Sevoflurane	P value
Age (years)	32.59 $\pm$ 8.917	30.36 $\pm$ 8.801	0.467 (NS)

\*significant  $P < 0.05$ , NS = non significant  $P > 0.05$

**Table (3): Student t-test showing comparison between type of inhalation anesthetic and operation period among studied cases.**

Type of anesthetic	Isoflurane	Sevoflurane	P value
Operation period (in hours)	2.064 $\pm$ 0.9045	1.136 $\pm$ 0.2335*	0.002 (NS)

\*significant  $P < 0.05$ , NS = non significant  $P > 0.05$ .

**Table (4): Distribution of RAPD PCR band changes after single exposure to inhalation anesthetic among studied cases.**

RAPD PCR Changes	N (%)
No change in bands	10 (20%)
Change in bands	40(80%)
Band loss and decreased intensity	11(22%)
Band gain and increased intensity	11(22%)
Combined band loss and gain	18 (36%)
Band gain	24(66%)
Band loss	19 (38%)
Increased intensity of bands	8 (16%)
Decreased intensity of bands	16 (32%)

*N*: number

**Table (5): Chi-square test showing comparison between the RAPD PCR results after single exposure to inhalation anesthetic and type of anesthetic among studied cases.**

Type of inhalation anesthetic	Isoflurane N (%)	Sevoflurane N (%)	P value
No change in bands	9 (23.1%)	1 (9.1%)*	0.004 (S)
Band loss and decreased intensity	11 (28.2%)	0 (0%)*	
Band gain and increased intensity	10 (25.6%)	1 (9.1%)*	
Combined band loss and gain	9 (23.1%)	9 (81.8%)*	

\*significant  $P < 0.05$ , *N*=number, *S*: significant

**Table (6): ANOVA test showing comparison between the RAPD PCR results after single exposure to inhalation anesthetic and the age among studied cases.**

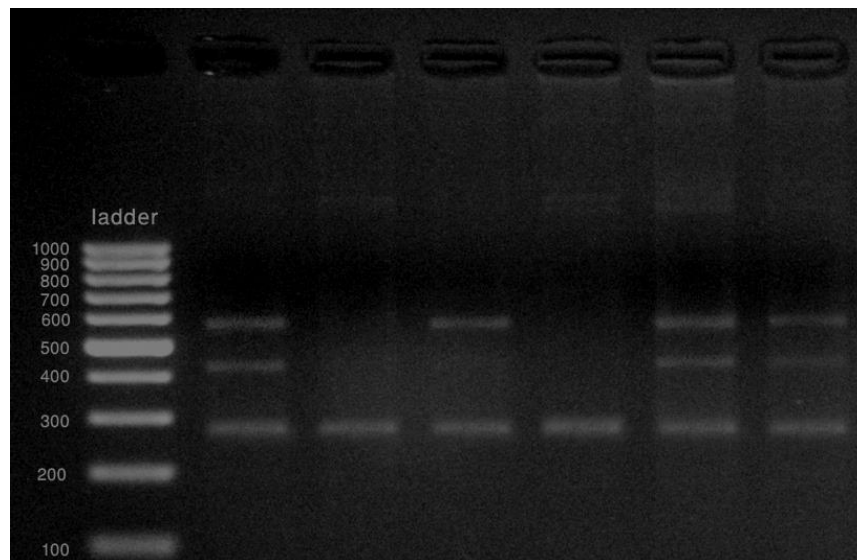
RAPD PCR changes	Age	P value
No change in bands	31.50 $\pm$ 9.664	0.752 (NS)
Band loss and decreased intensity	33.64 $\pm$ 8.262	
Band gain and increased intensity	29.82 $\pm$ 9.163	
Combined band loss and gain	32.89 $\pm$ 9.003	
Total	32.1 $\pm$ 8.851	

\*significant  $P < 0.05$ , NS: non significant  $P > 0.05$

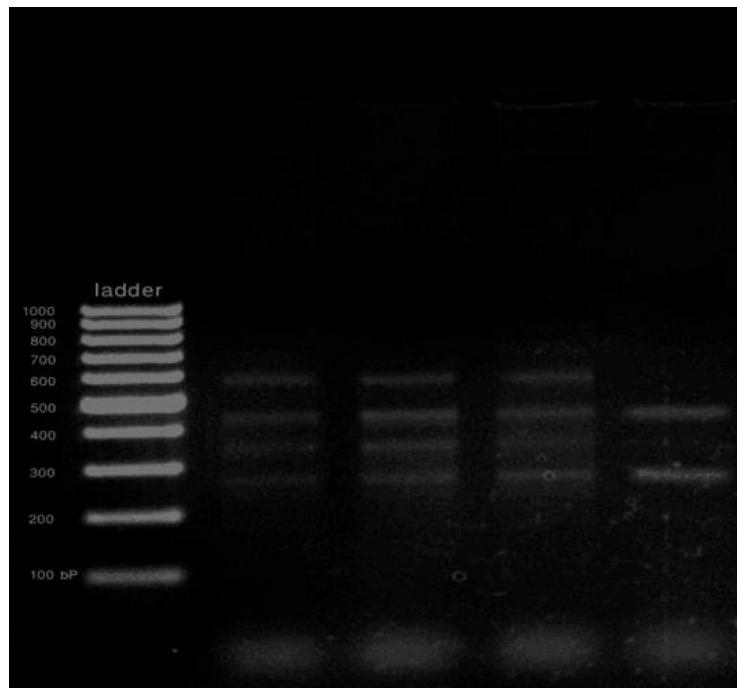
**Table (7): Chi-square test showing comparison between the RAPD PCR results after single exposure to inhalation anesthetic and gender among studied cases.**

RAPD PCR changes	Male N(%)	Female N(%)	Total N(%)	P value
No change in bands	5(25%)	5(16.7%)	10(20%)	0.343 (NS)
Band loss or decreased intensity	5(25%)	6(20%)	11(22%)	
Band gain or increased intensity	4(20%)	7(23.3%)	11(22%)	
Combined band loss and gain	6(30%)	12(40%)	18(36%)	

Significant  $P < 0.05$ , NS: non significant  $P > 0.05$ , N: number



**Figure (1): Photograph of DNA electrophoretic pattern of blood samples from patients receiving inhalation anesthesia using RAPD analysis :Lane 1 showing a molecular weight marker (DNA ladder). Lane 2 is the RAPD pattern of a patient before receiving inhalation anesthesia and lane 3 is the RAPD pattern of the same patient after receiving isoflurane showing 2 band loss. Lane 4 is the RAPD pattern of a patient before receiving inhalation anesthesia and lane 5 is the RAPD pattern of the same patient after receiving isoflurane showing one band loss . Lane 6 is the RAPD pattern of a patient before receiving inhalation anesthesia and lane 7 is the RAPD pattern of the same patient after receiving isoflurane showing decrease band intensity.**



**Figure (2):** Photograph of DNA electrophoretic pattern of blood samples from patients receiving inhalation anesthesia using RAPD analysis :Lane 1 showing a molecular weight marker (DNA ladder).Lane 2 is the RAPD pattern of a patient before receiving inhalation anesthesia and lane 3 is the RAPD pattern of the same patient after receiving isoflurane showing increase intensity of 3 bands. Lane 4 is the RAPD pattern of a patient before receiving inhalation anesthesia and lane 5 is the RAPD pattern of the same patient after receiving sevoflurane showing 2 bands loss and increase intensity of the other 2 bands.

## Discussion

The obtained results revealed that the exposure to inhalation anesthetics led to DNA changes observed as a difference in the RAPD PCR pattern of the patient before and after exposure to the anesthetic. Eighty percent of patients exposed to inhalation anesthesia for the first time had a change in the RAPD PCR pattern in the form of band gain, increased band intensity, band loss, or decreased band intensity.

Two inhalation anesthetics were used in the present study; isoflurane and sevoflurane. The present study showed that isoflurane was less mutagenic than sevoflurane. Studying the DNA lesions revealed that band changes showed significant statistical difference between the patients receiving isoflurane and those receiving sevoflurane. The vast majority of those received sevoflurane had combined band loss and gain (81.8%) compared to only 23.1% among those received isoflurane. This may be explained by the low level of metabolic breakdown of isoflurane (Dolores et al., 1997). When wistar rats exposed to either isoflurane or sevoflurane for 120 min, sevoflurane was found to induce DNA damage whereas isoflurane led to a higher antioxidative status (Rocha et al., 2015). However, about 5% of inhaled sevoflurane is metabolized in the liver by

cytochrome P450 2E1 isoenzyme, giving rise to reactive products, which could directly trigger the generation of peroxynitrite and increase peroxides and nitric oxide (Brozovic et al., 2008). It is known that free radicals or reactive oxygen species (ROS) are major oxidants that react with DNA, damaging it by various lesions, such as oxidized bases, abasic sites, and/or strand breaks (Cadet et al., 2003). Some authors have also suggested that fluorinated anesthetics, including sevoflurane, could directly lead to DNA damage, and the most probable modification would be an alkylation of purines (Jałoszyński et al., 1999).

Goldberg et al. (1996) found that sevoflurane biotransformation resulted in the generation of greater amounts of fluoride ions than isoflurane. As known, sevoflurane metabolism occurs predominantly in the liver by cytochrome P450 2E1 isoenzyme (Martin and Njoku 2005) but its extrahepatic metabolism is also present, which strongly influenced its nephrotoxicity (Erdem et al., 2006).

According to Braz et al. (2011)b exposure of patients to isoflurane had no genotoxic or cytotoxic effects in leukocytes, however, genetic repair and apoptosis-related genes were down-regulated on the first

post-operative day. Sevoflurane seems to be a more effective producer of oxidative stress than isoflurane. In sevoflurane treated human leukocytes, a dose-dependent oxidative stress and cellular injury, especially apoptosis, were observed (Matsuoka et al., 2001 and Wong et al., 2006).

Clinical studies indicated the absence of systemic DNA damage or oxidative DNA damage in patients under isoflurane anesthesia. In patients undergoing minimally invasive surgery under isoflurane anesthesia, a slight increase in the plasma's antioxidant capacity was observed (Braz et al., 2011)b.

In the present study patients subjected to ENT operations were included to verify the possibility of DNA changes within short duration of anesthetic exposure. Neurosurgical operations were mainly chosen as the expected long duration of operations would allow studying the anesthetic effect on DNA at largest exposure period. El-Banna et al. (2007) carried out their study on ENT and neurosurgical patients receiving isoflurane and sevoflurane.

In the present study, after taking control samples before induction of anesthesia, the second samples were collected after the end of operation to maximize the exposure period for anesthesia for detection of any possible genotoxic effect of the received anaesthetic. On the other hand Akin et al. (2005) has standardized the timing of the second sample, being after one hour from the start of the operation. El-Banna et al. (2007) applied RAPD assay on DNA extracted from blood samples collected in EDTA tubes from each patient before induction of anesthesia (as a control sample), just before the end of operation and 24 hours after induction of anesthesia.

RAPD analysis of the second samples of the studied patients revealed a variety of DNA lesions, which changed the DNA fingerprint pattern in the form of band gain, band loss or change in band intensity. The detection of these polymorphic changes turned the case positive for occurrence of DNA changes while the case was said to be negative on absence of these changes. Changes observed in RAPD profiles were scored by identifying the appearance and disappearance of bands compared to the control (Padmesh et al., 1999).

Bands disappear when the primer fails to bind to a certain site on the DNA that was altered by the genotoxic substance, while new bands appear when some sites on the DNA become accessible to the primer after structural change (insertions, deletions or breaks) by the genotoxic agent (Pietrasanata et al., 2000 and Enan, 2006).

However, it is very unlikely that mutations occur in a large portion of cells, because most of the DNA damage (which can lead to mutations during DNA replication) will be efficiently repaired. DNA lesions such as bulky adducts are expected to have detrimental effects on RAPD profiles. Not only can they induce structural changes, but they can also reduce the polymerization of the DNA and/or block the Taq DNA

polymerase (Nelson et al., 1996) which will result in a decrease in band intensity, or alternatively, in a disappearance of amplified products in the case of extensive DNA damage (Atienzar, 2000).

The least duration for DNA change to occur in the present study was recorded at one hour. El-Banna et al. (2007) recorded that the least duration for DNA change to occur was 0.75 hour.

As regard the relation between age of the studied patients and occurrence of DNA changes, the current study revealed that, although band loss was associated with higher age and band gain was associated with younger age, the differences were non significant. A similar observation was reported by (El-Banna et al., 2007). Also, Rozgaj et al. (2009) detected that age did not show any influence on the incidence of micronuclei among occupationally exposed persons.

Fenech (1993) observed vulnerability of elderly patients to DNA insult represented by higher frequency of micronuclei compared to middle age cases. They recorded that, in people 60 years of age; it was twice as high as in people 30 years of age. The accumulation of DNA damage is a significant event in the aging of cells. During this process, there is a progressive decrease in metabolic enzymes and DNA-repair enzymes, which increases the predisposition and susceptibility of the cells to exogenous and/or endogenous genotoxic agents. These factors contribute to an increase in age-related spontaneous DNA damage (Picerno et al., 2007).

As regard the relation between sex of the studied patients and occurrence of DNA changes, the current study revealed that females were more vulnerable to DNA changes however there is no significant relation between DNA changes and sex of the patients exposed to anesthesia which might be due to females predominance. The explanation for the difference in chromosomal damage between the nurses and the doctors may be the longer and higher exposure of the former group;. These results were consistent with the results recorded by El-Banna et al., (2007). This also is in agreement with Bonassi et al. (2011) who recorded that the effects of gender on the frequency of MN in exfoliated cells are not significant, unlike those observed with lymphocytes (where women tend to exhibit higher frequencies).

Fenech (1998) who found that there was a large inter-individual variability regarding micronucleus test where in the women, the micronucleus frequency was 1.4 times more than in men. Also, Rozgaj and Kasuba (2000) proved that sex was one of the factors influencing micronucleus assay as they found a statistically higher frequency of micronucleated cells among exposed females than males in either anesthesiologists or technicians exposed to anesthetic drugs. In contrast, Krause et al. (2003) found that there were no differences between male or female children as regards rate of occurrence of sister chromatid exchange. The difference in the genomic length of the X and Y chromosomes was considered the most likely cause of the different extent of chromosome damage between genders. The difference

between men and women might be not only due to different exposures but also to gender-related responses to mutagens. In particular, some chromosomal changes, specific genetic polymorphisms and epigenetic effects are plausible mechanisms explaining the gender-related differences.

As regard the relation between the DNA changes and operation period the present study show that band change was significantly related to the operation period; the shorter the operation period, the likely to show "no band change". Also, the maximum mean operation period was seen among those having band loss (2.59 hours) followed by band gain (2.14 hours) then combined band loss and gain (1.56 hours) and lastly no change (1.30 hours).

El-Banna et al. (2007) recorded to have DNA changes within 2.5 hours of exposure for sevoflurane and 3 hours for isoflurane with no statistical significance. Similiary, Szyfter et al. (2004) reported that the genotoxic effect of inhalation anesthetic was not apparently time-dependent.

In the present study, analysis of band polymorphism obtained by RAPD-PCR using selective primers, showed that the majority of patients suffered band gain (48%); followed by band loss (38%); then decreased band intensity (32%) and lastly increased band intensity (16%). A different observation was reported by El-Banna et al. (2007) who noticed that band loss was more frequent than band gain after patient exposure to anesthetic. A different observation was reported by Enan (2006) who noticed that band loss was more frequent than band gain after plant exposure to para-nitrophenol using arbitrary primed PCR.

## Conclusion

The results of the present study revealed that the exposure to inhalation anesthetics led to DNA changes observed as a difference in the RAPD PCR pattern of the patient before and after exposure to the anesthetic which may lead to increased morbidity.

## Recommendations

An intervention study with a large sample size would be needed before any definitive conclusions can be drawn.

It is recommended to examine patients after a period of exposure to inhalation anesthesia to indicate whether the DNA changes are permanent or temporary.

It is important to extend the research, since the results of the existing studies are rather controversial.

It is important to know that the protocols used for anesthesia are quite different, making the conclusions and comparisons challenging.

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## الملخص العربي

### تقييم الآثار السمية الوراثية لغازات التخدير بعد التعرض المفرد للمرضى باستخدام تقنية التضخيم العشوائي للحمض النووي متعدد الأشكال ( تقنية رابد )

ولاء أحمد علام و سهير علي محمد و أسماء محمد خلف أحمد<sup>١</sup> و عايدة عابدين محمود<sup>٢</sup> و وائل ألهم محمود<sup>٣</sup>

**المقدمة:** يتعرض الحمض النووي باستمرار لمجموعة متنوعة من العوامل البيولوجية والكيميائية والفيزيائية ، والتي قد تغير هيكلها، وتغير من وظيفتها. من بين المركبات الخارجية ، جذبت غازات التخدير، التي تستخدم عادة في إجراءات التخدير العامة، الانتباه بسبب القلق من احتمال أن يكون لها آثار السمية الوراثية.

**الهدف من العمل:** إن الهدف من هذه الدراسة هو تقييم التأثيرات السمية الجينية لاثنتان من أدوية التخدير الاستنشاقى الأكثر شيوعا (إيزوفلوران و سيفوفلوران) بعد التعرض الحاد باستخدام تقنية التضخيم العشوائي للحمض النووي متعدد الأشكال (RAPD).

**طريقة البحث:** أجريت هذه الدراسة في مستشفى جامعة سوهاج على ٥٠ مريضا، حيث خضع المرضى لعمليات جراحية مختلفة وتعرضوا للتخدير الاستنشاقى لأول مرة .

**النتائج:** كشفت النتائج التي تم الحصول عليها أن التعرض للتخدير الاستنشاقى أدى إلى تغييرات في الحمض النووي لوحظت بوجود اختلاف في نمط RAPD PCR للمريض قبل وبعد التعرض للتخدير. ثمانون في المئة من المرضى المعرضين للتخدير الاستنشاقى للمرة الأولى لديهم تغيير في نمط RAPD PCR في شكل كسب الفرقة ، زيادة شدة الفرقة ، فقدان الفرقة ، أو انخفاض شدة الفرقة. حيث أظهرت الدراسة الحالية أن الأيزوفلورين كان أقل طفرات من سيفوفلوران .

**الخلاصة:** التخدير الاستنشاقى كان له تأثير سام على الجينات تم اكتشافه بواسطة تقنية RAPD-PCR .

**التوصيات:** يوصى بفحص المرضى بعد فترة من التعرض للتخدير الاستنشاقى لبيان ما اذا كانت التغيرات في الحمض النووي دائمة او مؤقتة . من المهم

توسيع نطاق البحث باستخدام عينه كبيرة وبروتوكولات مختلفة من التخدير .

١ قسم الطب الشرعي والسموم الإكلينيكية، كلية الطب البشرى، جامعة سوهاج، سوهاج، مصر.

٢ قسم الكيمياء الحيوية، كلية الطب البشرى، جامعة سوهاج، سوهاج، مصر.

٣ قسم التخدير، كلية الطب البشرى، جامعة سوهاج، سوهاج، مصر.