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ORIGINAL ARTICLE

High concentrations of waste anesthetic gases induce genetic damage and inflammation in physicians exposed for three years: A cross-sectional study

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Abstract

This cross-sectional study analyzed the impact of occupational waste anesthetic gases on genetic material, oxidative stress, and inflammation status in young physicians exposed to inhalational anesthetics at the end of their medical residency. Concentrations of waste anesthetic gases were measured in the operating rooms to assess anesthetic pollution. The exposed group comprised individuals occupationally exposed to inhalational anesthetics, while the control group comprised individuals without anesthetic exposure. We quantified DNA damage; genetic instability (micronucleus-MN); protein, lipid, and DNA oxidation; antioxidant activities; and proinflammatory cytokine levels. Trace concentrations of anesthetics (isoflurane: 5.3 ± 2.5 ppm, sevoflurane: 9.7 ± 5.9 ppm, and nitrous oxide: 180 ± 150 ppm) were above international recommended thresholds. Basal DNA damage and IL-17A were significantly higher in the exposed group [27 ± 20 a.u. and 20.7(19.1;31.8) pg/mL, respectively] compared to the control group [17 ± 11 a.u. and 19.0(18.9;19.5) pg/mL, respectively], and MN frequency was slightly increased in the exposed physicians (2.3-fold). No significant difference was observed regarding oxidative stress biomarkers. The findings highlight the genetic and inflammatory risks in young physicians exposed to inhalational agents in operating rooms lacking adequate scavenging systems. This potential health hazard can accompany these subjects throughout their professional lives and reinforces the need to reduce ambient air pollution and consequently, occupational exposure.

KEYWORDS
genomic instability, indoor air pollution, inflammation, inhalation anesthetics, oxidative stress, work environment

Braz and Carvalho equally contributed to this study.

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1 | INTRODUCTION

The Occupational Safety and Health Administration (OSHA) estimated that over 250 000 healthcare professionals are at risk of occupational illness due to chronic exposure to waste anesthetic gases.[1] Therefore, awareness of the adverse effects related to waste anesthetic gases has been a major topic for decades, and the concern is to ensure the health and minimize the risks of exposed individuals.[2] Fatigue, headache, irritability, nausea, drowsiness, and neurobehavioral impairment have been reported in exposed operating room staff.[3] Reproductive problems and hepatic diseases have also been linked to long-term waste anesthetic gas exposure.[4-8]

Human biomonitoring is a valuable tool for evaluating genetic and chromosomal damage in individuals who have been exposed to genotoxic and carcinogenic agents.[9] The comet and micronucleus (MN) assays, biomarkers of effects, are widely used to assess genotoxic and mutagenic risks,[10] which include occupational exposure to waste anesthetic gases.[11] Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses,[12] and changes in immune parameters have also been linked to long-term waste anesthetic gas exposure.[13-18]

Therefore, various studies on the hazards of occupational exposure to waste anesthetic gases have been published but have focused on chronic exposure, which means that the operating room personnel have been exposed for several years or decades. However, the impact of a shorter exposure to anesthetic gases, especially in young adult physicians at the end of their medical residency program, remains unknown. Thus, the current study monitored the concentrations of waste anesthetic gases in the ambient air of surgical theaters and evaluated the effects of occupational exposure to the most widely used inhalational anesthetics on genetic material, oxidative stress, and inflammatory status in physicians exposed for a 3-year period.

Practical Implications

- Worldwide, millions of professionals who work in operating rooms are occupationally exposed to inhalational anesthetics.
- High concentrations of waste anesthetic gases were found due to a lack of adequate ventilation and scavenging systems, which represent the reality for underdeveloped and developing countries.
- A high exposure to waste anesthetic gases in young adults showed deleterious effects at systemic and molecular levels.
- Better scavenging and ventilation systems in surgical theaters are urgently needed and would reduce waste anesthetic gas occupational exposure.

2 | METHODS

2.1 | Ethics and study design

This cross-sectional study was conducted after the approval of the Institutional Review Board (IRB) at Botucatu Medical School (4440-2012) and registration at www.ensaiosclinicos.gov.br (RBR-78m24n). The study was conducted in accordance with the World Medical Association Declaration of Helsinki regarding ethical conduct in research involving human subjects, and the paper was prepared in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement.[19]

2.2 | Study population

Sixty-three medical residents at the Botucatu Clinics Hospital (HCFMB), an institution that is fully affiliated with Botucatu Medical School—São Paulo State University (UNESP) in Brazil, were recruited at the end of their medical residency programs (third year). Medical residents who worked in the operating rooms and were exposed to isoflurane and sevoflurane (inhalational anesthetics) and nitrous oxide (anesthetic gas; commonly known as laughing gas) were recruited from Surgery (pediatric surgery; neurosurgery; ear, nose and throat; and ophthalmology) and Anesthesiology fields and composed the exposed group (n = 32), while residents from Internal Medicine who were not exposed to waste anesthetic gases made up the control group (n = 31). The groups were matched by age, sex, and body mass index. All participants signed a written informed consent form and answered a questionnaire regarding demographic data, lifestyle, and occupational information. The exclusion criteria included pregnancy, smoking, heavy drinking, illicit substance abuse, and previous radiotherapy or chemotherapy treatments. Subjects with any disease, those receiving any medical treatments and those taking antioxidant supplements were also excluded.

2.3 | Biological sample collection

Blood and buccal cell samples were concomitantly obtained from both groups at one time at the end of their medical residency. The samples were always obtained during the same period to avoid possible bias. Blood samples were collected in EDTA-containing evacuated tubes and in serum-separating tubes, promptly centrifuged (300 *g*, 15 minutes, 4°C) and aliquoted for lymphocyte isolation (comet assay) or stored as plasma and serum aliquots at −80°C until analysis. All samples were coded, protected from light, and analyzed in a blinded manner. Measurements of DNA damage and oxidative stress were performed under indirect light, and samples from the control and exposed groups were always mixed in each batch of assays. All experiments were run in duplicate or triplicate.

All assays involving plasma samples were conducted at the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA),

Tufts University, Boston, MA, USA. An unknown plasma sample was used for quality control (QC) throughout the assays. Coefficients of variation (CV) were calculated intra-assay (for the same experimental day) and inter-assay (for different experimental days). CV values below 5% were admitted. All standard curves used to calculate the results for unknown samples had R2 ≥ 0.99.

2.4 | Genotoxicity and mutagenicity assays

Genetic damage evaluation (basal DNA damage and oxidized bases) was conducted in lymphocytes that were isolated from freshly collected blood, and the comet assay was performed according to previously described protocols.[20,21] Briefly, cells were added to low-melting-point agarose at 37°C and set on slides, and the agarose was allowed to solidify. The slides were then immersed in a cold lysis solution. Oxidative damage was evaluated using the enzymes formamidopyrimidine-DNA glycosylase (FPG) and endonuclease III (endo III; New England Biolabs, USA) to detect oxidized purines and pyrimidines, respectively. Slides were subsequently washed with phosphate-buffered saline (PBS) and with enzyme buffer and incubated with FPG and endo III solution in a humidity chamber at 37°C for 30 minutes. A similar procedure was performed with control slides (only buffer without enzyme) for evaluation of basal DNA damage. The slides were then refrigerated and placed into a horizontal electrophoresis tank filled with cold, freshly prepared alkaline buffer for 40 minutes. Electrophoresis was performed (25 V, 300 mA, 30 minutes), and the slides were neutralized with 0.4 M Tris. Negative and positive controls were used during the experiments. Slides were stained with SYBR Gold and examined under a fluorescence microscope at 400× magnification. Images of 100 randomly selected nucleoids (50 from each of two replicate slides) per type of damage/per subject were analyzed using Comet Assay IV software (Perceptive Instruments, UK). Because the tail moment results were similar, only the tail intensity values were presented.

The MN assay was performed on buccal epithelial cells that were collected by rubbing the inside of the subject's cheeks with a spatula, which was then placed in a tube containing PBS. The cells were immediately processed and centrifuged, and the supernatant was aspirated. A fixing solution of methanol:acetic acid (3:1) was added, and the tubes were centrifuged once more. This step was repeated two more times. Using a pipette, the cell suspension was transferred to appropriately labeled microscope slides (duplicates) and left to air-dry for 24 hours. Next, the slides were immersed in HCl at 37°C for 20 minutes to induce hydrolysis and were then washed three times with Milli-Q water. The slides were then placed into another jar containing Schiff's reagent (Feulgen) for 90 minutes in the dark at room temperature, and the reagent was stirred for approximately 2 minutes at 30-minute intervals. The Feulgen stain was then removed, and the jar was washed in Milli-Q water. Then, the slides were allowed to air-dry for approximately 12 hours. After this period, the slides were immersed in cold Fast Green for 10 seconds, washed for another 10 seconds with ethanol, and air-dried. MN, a genomic instability biomarker, was evaluated under an optical microscope (Olympus BX43, Japan; 400× magnification) for 2000 differentiated cells per individual, to evaluate its frequency, according to previous recommendations.[22]

2.5 | Oxidative stress and inflammatory markers

Measurement of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) was conducted in plasma with liquid chromatography-tandem mass spectrometry (LC-MS/MS) using 15N7-8-oxo-dG as the internal standard.[23] Plasma sample ions were diluted with formic acid and subjected to solid-phase extraction using an Oasis HLB cartridge, which was activated with methanol and formic acid. The eluent was dried under N_2, reconstituted in dissolving buffer (5% acetonitrile-ACN in 1% aqueous formic acid), vortexed, sonicated, filtered, and injected into an AB SCIEX™ API 5500 LC-MS/MS (USA) system operating in positive ion mode. The LC-MS/MS instrumentation included an Agilent UHPLC (USA) coupled to a turbospray AB SCIEX™ API 5500 MS (USA) and an Agilent Zorbax Eclipse Plus C18 Rapid Resolution HD 2.1 × 50 mm 1.8 micron (USA). Mobile phase A (MPA) was 0.1% formic acid in water, and mobile phase B (MPB) was 0.1% formic acid in ACN. 8-oxo-dG was eluted in isocratic mode using 95% MPA and 5% MPB over 3 min at 35°C with a flow rate of 0.35 mL/min.

Lipid peroxidation was detected in plasma using two biomarkers: malondialdehyde (MDA) and 4-hydroxynonenal (HNE). MDA was analyzed after derivatization with thiobarbituric acid (TBA) and separation using high-performance liquid chromatography (HPLC) according to a previously described method.[24] Briefly, the samples were added to orthophosphoric acid and TBA. Following 1 hour of incubation at 100°C, the samples were placed on ice, and NaOH:methanol was added. The samples were then vortexed and centrifuged, and the supernatant was filtered and injected into an HPLC system. MDA was monitored at 532 nm. HNE was measured in plasma using an OxiSelect™ HNE Adduct Competitive ELISA Kit (Cell Biolabs Inc) according to the manufacturer's instructions.

Protein oxidation in plasma was assessed using an OxiSelect™ Protein Carbonyl ELISA Kit (Cell Biolabs Inc). Protein carbonyl content was adjusted for total plasma protein, which was determined using a Pierce™ BCA Protein Assay Kit (Thermo Scientific). Serum inflammatory interleukin 17A (IL-17A) was analyzed by flow cytometry using the BD™ CBA Human IL-17A Enhanced Sensitivity Flex Set (BD Biosciences).

2.6 | Antioxidant assays

Antioxidant capacity in plasma was assessed using the Ferric Reducing Ability of Plasma (FRAP) and Aqueous Oxygen Radical Absorbent Capacity Assay (ORAC) with perchloric acid (PCA) precipitation.

The FRAP assay measures the capacity of plasma antioxidants to reduce the ferric ion (Fe3+) to the ferrous ion (Fe2+) in the presence of 2,4,6-tripyridyl-S-triazine (TPTZ). The assay was performed using a spectrophotometric method (Shimadzu UV-1601 reader).[25,26]

The ORAC-PCA plasma protocol was performed following a previously described method.[27] Plasma antioxidants compete with the fluorescent probe fluorescein to reduce peroxyl radicals that are generated from the radical initiator 2,2′-azobis-(amidinopropane) dihydrochloride [AAPH]. A FLUOstar OPTIMA plate reader (BMG Labtech) was used to monitor the fluorescence intensity at 485 nm excitation and 520 nm emission.

The Total Antioxidant Performance (TAP) assay was also conducted according to a previously described protocol[28] with minor modifications.[29] This method measures the lipid compartment oxidizability of human plasma using the lipophilic radical initiator 2,2′-azobis (4-methoxy-2,4-dimethylvaleronitrile) [MeO-AMVN] and the fluorescent probe BODIPY 581/591. The fluorescence was determined by the oxidation product of BODIPY in a multilabel counter (Wallac Victor 2; Perkin Elmer Life Sciences).

2.7 | Waste anesthetic concentrations in operating rooms

Botucatu Clinics Hospital is a tertiary teaching facility with a surgical theater that provides elective and urgency multispecialty procedures, where half of the operating rooms have scavenging systems that are limited to 25% fresh air with 7 air changes/h and the other half have no anesthetic ventilation or scavenging systems. All exposed medical residents worked in all operating rooms. Real-time waste anesthetic gas concentrations (isoflurane, sevoflurane, and nitrous oxide) were measured near the medical residents' breathing zones during all surgeries when inhalational anesthesia was administered in the last 6 months of exposure during the third year of the medical residency using a portable infrared vapor analyzer (InfraRan 4-Gas Anesthetic Specific Vapor Analyzer, Wilks Enterprise) to assess the anesthetic pollution. The specific analyzer can detect up to 100 parts per million (ppm) for halogenated anesthetics and 400 ppm to nitrous oxide; the recommended measurement ranges are 0-50 ppm for halogenated anesthetics (isoflurane and sevoflurane) and 0-100 ppm to nitrous oxide. Prior to performing each measurement, a trained operator calibrated the device according to the manufacturer's recommendations. Fresh gas flow (FGF) of 1-2 L/min was used during the maintenance of anesthesia as routinely utilized in the surgical theater. The reported concentrations were expressed as ppm. The means were calculated for all waste anesthetic gases, and the time-weighted average (TWA) was also reported for nitrous oxide.

2.8 | Statistical analyses

The results were expressed as frequencies, means and standard deviations (X ± SD), or medians and quartiles. The qualitative variable (sex) was analyzed using the chi-square test. Quantitative variables following the normal distribution were analyzed using Student's *t* test, and a nonparametric test (the Mann-Whitney test) was applied otherwise. MN was evaluated using the Poisson regression model. Differences with *P* values < .05 were considered significant.

3 | RESULTS

The data regarding waste anesthetic gas concentrations in operating rooms are shown in Table 1. The operating rooms without a scavenging system had much higher trace concentrations of anesthetic than the operating rooms equipped with a scavenging system. The mean concentrations of the anesthetic (nitrous oxide > sevoflurane > isoflurane) contributed to operating room air pollution.

Table 2 shows the demographic data of the subjects; there were no differences between the groups since they were matched. The subjects in the exposed group worked for 37 h/wk in the operating rooms while the control group worked for 35 h/wk in ambulatory care clinics.

The comet assay revealed a significant increase (1.6-fold) in basal DNA damage in the exposed group (27 ± 20 a.u.) compared to the control group (17 ± 11 a.u.) (Figure 1; *P* = .01). Buccal MN frequency was increased (2.3-fold) in the exposed group (0.7 ± 1.0/2000 cells); compared to the control group (0.3 ± 0.7/2000 cells), but the difference

TABLE 1 Concentrations (ppm) of halogenated anesthetics and nitrous oxide in operating rooms

Anesthetic	Scavenging system in operating rooms		Mean	Recommended exposure limits[b]
	No	Yes		
Isoflurane	9.2 (3.0-17.8)	1.3 (0.3-3.2)	5.3	2
Sevoflurane	16.4 (5.3-34.1)	2.9 (1.0-7.2)	9.7	2
Nitrous oxide	235 (120-350)	66 (61-70)	180[a]	25[a]

Note: Data expressed as mean, minimum, and maximum ranges.

[a]Time-weighted average (TWA) according to administration time.

[b]according to the National Institute for Occupational Safety and Health (NIOSH).

TABLE 2 Demographic data

Characteristic	Control Group (n = 31)	Exposed Group (n = 32)
Age (years)	27.4 ± 1.6	28.7 ± 1.9
Sex (male/female)	19/12	20/12
Body mass index (kg/m^2)	24.5 ± 3.9	24.6 ± 3.8

Note: Data expressed as X ± SD or absolute number. $P > .05$.

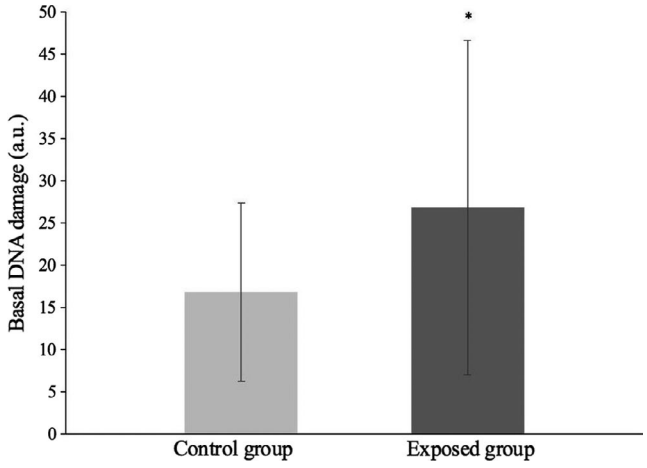

FIGURE 1 Basal DNA damage (X ± SD) evaluated using the comet assay in peripheral lymphocytes in control and exposed groups; *P = .01

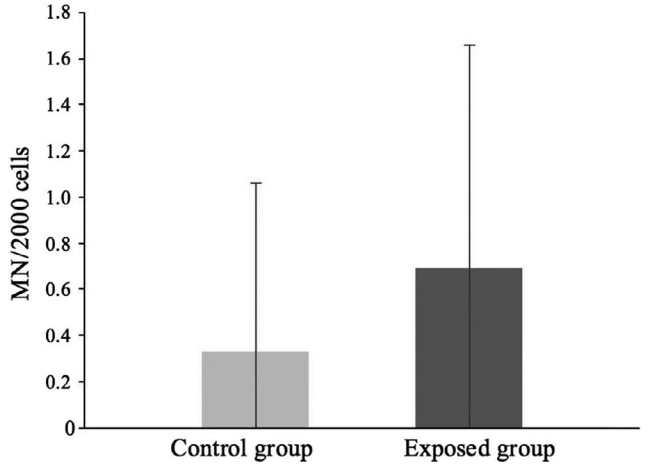

FIGURE 2 Frequency (X ± SD) of micronucleus (MN) in buccal cells in control and exposed groups; P = .07

between the groups was not significant (Figure 2; P = .07). IL-17A was significantly increased in the exposed [20.7 (19.1;31.8) pg/mL] group compared to the control group [19.0 (18.9;19.5) pg/mL] (Figure 3; P = .03).

Table 3 shows the oxidative stress biomarkers and plasma antioxidant assay results; there were no statistically significant differences between the groups.

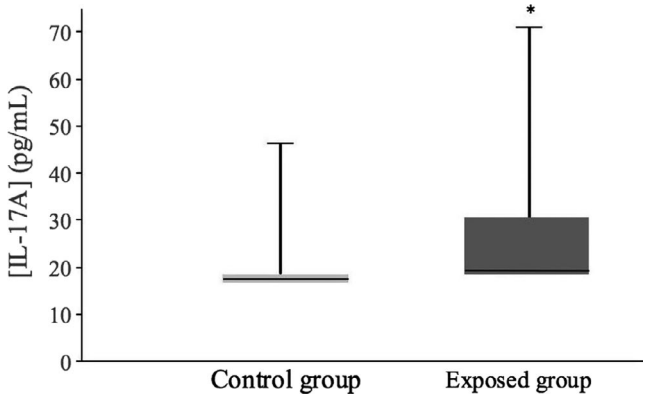

FIGURE 3 Interleukin 17A concentrations in control and exposed groups (median and quartiles); *P = .03

TABLE 3 Oxidative stress biomarkers: DNA, lipids and protein, and antioxidant capacities

Parameter	Control group	Exposed group	*P* value
DNA			
Oxidized purines (a.u.)	35.5 ± 34.0	34.3 ± 34.5	.9
Oxidized pyrimidines (a.u.)	33.8 ± 26.2	44.8 ± 37.9	.2
8-oxo-dG (pg/mL)	0.43 ± 0.31	0.48 ± 0.52	.7
Lipids			
MDA (μmoL/L)	0.60 ± 0.21	0.61 ± 0.24	.8
HNE (μg/mL)	8.7 [5.0; 12.5]	9.3 [4.4; 14.1]	.7
Protein			
Protein carbonyl (nmoL/mg)	4.4 ± 1.8	4.5 ± 1.7	.9
Antioxidant capacities			
FRAP (μmoL/L)	381.7 [304.7; 500.6]	442.0 [335.0; 691.7]	.2
ORAC (μmoL/L)	1567.8 ± 388.6	1611.2 ± 396.4	.7
TAP (%)	77.5 ± 6.2	79.9 ± 7.8	.4

Note: Data expressed as X ± SD or median and quartiles.

Abbreviations: 8-oxo-dG, 8-oxo-2′-deoxyguanosine; FRAP, Ferric Reducing Ability of Plasma; HNE, 4-hydroxynonenal; MDA, malondialdehyde; ORAC, Oxygen Radical Absorbance Capacity; TAP, Total Antioxidant Performance.

4 | DISCUSSION

In the current study, we observed increased genetic damage and inflammatory cytokine levels, but no changes in oxidative stress markers, in physicians exposed for 3 years to trace amounts of isoflurane, sevoflurane, and nitrous oxide in the operating rooms without adequate ventilation and scavenging systems.

Medical residents are a special category of physicians whose occupational and personal well-being are of major concern worldwide.[30] Controversial findings have already been reported regarding genotoxicity observed after a few months of exposure to waste anesthetic gases.[31,32] In the aforementioned papers, a study[31] had already reported increased DNA damage in physicians exposed for eight months to inhalational anesthetics in operating rooms without any scavenging systems compared to those in a nonexposed group. In addition, the physicians used far more inhalational anesthetics than intravenous anesthetics, and the anesthesia equipment was older; both factors certainly contributed to high levels of DNA damage, and no measurement of anesthetic concentrations in the workplace was performed. In contrast, physicians exposed to inhalational anesthetics for up to 12 months using only modern anesthesia equipment did not show an increase in DNA damage.[32] The present study showed increased DNA damage (detected as single- and double-strand breaks and alkali-labile sites) in medical residents exposed for 36 months in operating rooms without adequate scavenging/ventilation systems, which certainly contributed to the high levels of waste anesthetic gas pollution and consequent genetic damage. In fact, the comet assay is a reliable genotoxicity biomarker that is widely used for human biomonitoring and exhibits high sensitivity for detecting DNA damage.[9] Thus, our findings emphasize that this assay is an important genotoxicity tool that can be utilized to detect early alterations in the genome of young physicians with high exposure levels to trace amounts of anesthetics.

DNA lesions can be repaired or fixed, which may lead to genomic instability.[33] The buccal MN assay is a minimally invasive method that monitors mutagenic events by detecting DNA damage and chromosomal instability.[22] Increased MN formation may be associated with early carcinogenic events.[34] Although we did not observe a statistically significant enhancement of MN formation in the exposed group, the medical residents who were exposed to waste anesthetic gases had a MN frequency that was more than double that of unexposed residents, suggesting its biological importance. Consistent with these results, we also previously observed that anesthesiologists working in the same studied hospital who were chronically exposed (average of 16 years) to waste anesthetic gases had a significantly higher buccal MN frequency (2.3-fold) compared to unexposed physicians.[35] Another study[36] also reported elevated buccal MN frequency (2.6-fold) in operating room personnel after chronic exposure to older (enflurane and halothane) and modern (isoflurane, sevoflurane, and desflurane) halogenated anesthetics together with nitrous oxide. Therefore, the slightly increased MN frequency observed in the studied medical residents might result in genomic instability over time.

Although mechanisms by which halogenated anesthetics induce genotoxicity remain to be elucidated, plausible explanations include the production of ROS due to oxidation metabolism and/or direct genome damage.[18,37,38] Furthermore, nitrous oxide-induced impairment of methionine synthase affects nucleic acid and protein production, which in turn reduces genomic stability.[39]

The evidence regarding the genotoxic and mutagenic effects of chronic (long-term) occupational exposure to waste anesthetic gases that has been reported in the literature is controversial.[17,36,40,41] The inconsistency in the results may be attributed to differences in a variety of factors, for example, the studied populations, the surgical theaters involved, the use of anesthetic agents, and the lack of measurement or differences in the waste anesthetic gas concentrations found in the operating rooms.

The largest difference between long-term exposure and that in our study (shorter exposure) is the time of exposure. We evaluated a 3-year exposure period since the physicians were at the end of their specialization. In contrast to the majority of studies in the literature that reported DNA damage biomarkers in exposed professionals who worked for several years or decades, we evaluated only healthy young adult physicians who worked at the same workplace, at the end of their medical residency program. On the other hand, professionals exposed for a long period may have some comorbidities that may influence the biomarker data. Therefore, our goal was to understand whether these young adults already have changes in important biomarkers during their residency period. Thus, this study tried to contribute, for the first time in the literature, to a better understanding of the possible association between occupational exposure to anesthetics and genetic damage, oxidative stress and inflammatory markers in medical residents at the end of their specialization.

Enhanced oxidative stress and the oxidative modification of nucleic acids, lipids and proteins contribute to the pathogenesis of many diseases, including cardiovascular and neurological diseases and cancer.[13] A growing interest in the link between oxidative stress and chronic occupational exposure to waste anesthetic gases has arisen.[16,17,42,43] Given the scarce evidence obtained thus far and the uncertainty in the alterations caused by a shorter exposure to waste anesthetic gases, it was important to evaluate whether oxidative stress could play a role in the deleterious effects of volatile anesthetics on operating room professionals.

In addition to DNA breaks, a range of possibilities exists for detecting oxidative DNA damage using the alkaline comet assay (determined here as oxidized purines and pyrimidines) and by 8-oxo-dG. Protein oxidation can be evaluated by measuring the levels of protein carbonyl groups (early and stable compounds produced by ROS in amino acid chains).[44] Additionally, lipid peroxidation can induce the destruction of membranes through the production of reactive aldehydes; HNE and MDA are the most frequently studied due to their stability and biological activity as the second messengers of free radicals.[45] Moreover, various methods for assessing antioxidant activity exist. However, our current report is the only study to perform three different antioxidant activity assays in both hydrophilic and lipophilic compartments, thus obtaining a broad view of the subjects' antioxidant statuses. No change in antioxidant capacities was observed in these young physicians who were exposed to waste anesthetic gases, and this observation is in accordance with a previous study that evaluated FRAP data in chronically exposed personnel.[42] Although a

comprehensive analysis of oxidative biomarkers was conducted, it appears that a 36-month exposure to waste anesthetic gases is not associated with enhanced oxidative stress when assessed in young and healthy medical residents. A possible hypothesis is that oxidative stress could occur earlier to DNA damage.

A well-established connection exists between oxidative stress and inflammation. Inflammatory processes attract leukocytes to injury sites and increase oxygen uptake, thus creating a respiratory burst that results in the local accumulation of ROS. A "vicious circle" may then arise as inflammatory cells produce mediators that recruit further inflammatory cells, producing yet more ROS.[46] However, reports on immune parameters in professionals exposed to waste anesthetic gases remain lacking in the literature.[8,13,14] Our study illustrated that IL-17A levels were elevated in medical residents after exposure to waste anesthetic gases. IL-17A plays a key role in innate and adaptive immune responses, and this potent proinflammatory cytokine is associated with respiratory disorders.[47] Interestingly, previous data revealed increased proinflammatory IL-8 levels in young physicians exposed to waste anesthetic gases.[48] Because both IL-8 and IL-17 are inflammatory cytokines that play roles in the human airways, the increases in levels of these two markers illustrate the relevant impact of waste anesthetic gas inhalation.

For waste anesthetic gas exposure, the National Institute for Occupational Safety and Health (NIOSH) has published recommendations concerning adequate working conditions, the use of scavenging/ventilation systems, and the reduction of occupational exposure by limiting the trace concentrations of waste anesthetic gases. The recommended exposure limits (REL) were set at 2 ppm for halogenated anesthetic and at 25 ppm for nitrous oxide (TWA during anesthetic administration).[49] These standards vary among countries, but formal regulation and safety measures are lacking in many developing nations, such as Brazil.[50] We therefore opted to use NIOSH's REL as a reference, even though we are aware that these recommendations do not consider modern halogenated anesthetics, such as isoflurane and sevoflurane.[2] The mean concentrations of all waste anesthetic gases detected in our operating rooms were above the REL and were higher in operating rooms without a scavenging system. The mean nitrous oxide values exceeded the NIOSH recommendations[49] by 7.2-fold, highlighting an alarming situation.

Importantly, in the operating rooms that were equipped with scavenging systems, the number of air exchanges per hour did not fulfill the standards of the American Institute of Architects, which recommends 15-21 exchanges/h to ensure complete external fresh room air.[51] Furthermore, half of the operating rooms continue to function without scavenging systems because they are part of an older aisle of the surgical theater and unfortunately reflect the reality of millions of operating rooms worldwide, especially in less-developed countries. Note that while modern scavenging systems have minimized the impact of waste anesthetic gases in high-income countries, several centers around the world, including those in underdeveloped and developing countries, do not have access to quality scavenging and ventilation,[52,53] thereby bringing the issue into sharp focus. The trace concentrations of waste anesthetic gases that were measured in this study not only reveal the importance of having proper scavenging and ventilation systems in operating rooms but also clarify the need for regular maintenance and constant monitoring of air quality to prevent, identify and/or correct eventual leakages and malfunctions.[2] It is equally critical to continuously educate and train all healthcare workers to adopt safety measures that contribute to reducing operating room air pollution.[1]

One limitation of our study is that the medical residents who worked in the operating rooms were sporadically exposed to radiation, although proper protection was used (cervical lead shield and lead apron) to minimize the exposure. Furthermore, to avoid possible bias, medical residents involved in orthopedic and vascular surgeries were not included due to their daily radiation exposure. Additionally, we followed rigorous standards regarding the measurement of waste anesthetic gases, the inclusion and exclusion criteria used, the use of properly matched groups, and sample and data blinding.

In conclusion, the high waste anesthetic gas concentrations observed in operating rooms lacking adequate scavenging systems are associated with genomic damage and an inflammatory state but not oxidative stress in young medical residents exposed to a 36-month period. This potential health hazard can accompany these subjects throughout their professional lives and reinforces the need to adopt adequate measures to diminish ambient air pollution. It is critical that all operating room personnel, particularly young personnel, are aware of this occupational exposure once this is a public health issue.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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