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RESEARCH ARTICLE

Effects of occupational exposure to trace levels of halogenated anesthetics on the liver, kidney, and oxidative stress parameters in operating room personnel

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ABSTRACT

This study was conducted to measure the concentration of halogenated anesthetics in breathing zone air of the personnel and evaluate their effects on hepatic and renal functions as well as oxidative stress parameters. Levels of these gases in air samples were lower than the national recommended exposure limits (50 ppm). Occupational exposure to anesthetic gases significantly induced oxidative stress in the operating room personnel, marked by increased plasma levels of transaminase enzymes accompanied by the marked decline of enzymatic and non-enzymatic antioxidant levels, and the rise of MDA levels ($p < 0.05$); these adverse effects would increase with increasing work experience.

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Introduction

Several inhalational anesthetic agents are being used to induce and maintain anesthesia. Even in modern operating rooms (ORs), the trace levels of volatile anesthetics may leak from the breathing circuit and pollute the ambient air. Contamination of the OR environment occurs due to several reasons, including induction of anesthesia, pediatric anesthesia, exhalation of the patient, anesthesia machine leakage, inadequate scavenging system, and so on (Hoerauf *et al.* 1996; Irwin *et al.* 2009, Jafari *et al.* 2018). Thus, OR personnel are unavoidably exposed to inhalational anesthetics that may lead to adverse health effects (Byhahn *et al.* 2001, Irwin *et al.* 2009). According to previous experimental studies, long-term exposure to anesthetic gases, especially halogenated agents (e.g. isoflurane, sevoflurane, enflurane, and methoxyflurane), can result in genotoxicity, neurotoxicity, spontaneous abortion, congenital malformations, as well as liver and kidney damage (McGregor 2000, Grasshoff and Antkowiak 2006, Rocha *et al.* 2015, Zhu *et al.* 2017). Although the exact mechanisms underlying these effects have not been elucidated, some researchers believe that anesthetic agents exert their adverse effect via inducing of oxidative stress (Malekirad *et al.* 2005, Türkan *et al.* 2005). Oxidative stress is defined as a marked imbalance between the production of reactive oxygen species (ROS) and antioxidant defense. Based on previous investigations, chronic exposure to anesthetic gases induces the ROS formation and oxidative damage to macromolecules (i.e. DNA, proteins, and lipids; Ranjbar *et al.* 2007, Irwin *et al.* 2009).

However, results of epidemiological studies are still controversial. Some researchers believe there is no causal relationship between exposure to trace concentrations of inhalational anesthetic gases and the possible development of adverse health effects (McGregor 2000, Hansen 2015). However, some other investigators have reported that long-term occupational exposure to low levels of these gases has adverse effects on the health of OR personnel (Byhahn *et al.* 2001, Costa Paes *et al.* 2014). In contrast to halothane, which causes liver injury and induces teratogenic effects (Neuberger *et al.* 1981, Byhahn *et al.* 2001, Safari *et al.* 2014), there is no consensus about the adverse effects of other anesthetic agents in spite of abundant literature in this regard (American Society of Anesthesiologists, 1974, Cohen *et al.* 1975, Frankhuizen *et al.* 1978, Byhahn *et al.* 2001, Molina Aragonés *et al.* 2016). Nonetheless, the possible health

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hazards from occupational exposure to other halogenated anesthetics cannot be completely ruled out (Molina Aragonés *et al.* 2016). That is why some health authorities recommend occupational exposure limits for anesthetic gases to minimize their possible adverse health effects. For instance, the American Conference of Governmental Industrial Hygienists (ACGIH) has recommended the threshold limit value (TLV) of 50 ppm for halothane and nitrous oxide (N_2O) and 75 ppm for enflurane (American Conference of Governmental Industrial Hygienists 2017). Further, the USA National Institute for Occupational Safety and Health (NIOSH) proposed that the 8-h time-weighted average (TWA) concentration of N_2O should not exceed 25 ppm and all the halogenated agents should be less than 2 ppm if used alone, or below 0.5 ppm when used in combination with N_2O (National Institute for Occupational Safety and Health 1977). In European countries, however, the recommended values for halogenated volatile agents range from 2 to 50 ppm over an 8-h working day (Byhahn *et al.* 2001, González García *et al.* 2001). Ministry of Health and medical Education in Iran also has a limit of 50 ppm for N_2O, halothane, and isoflurane, and 20 ppm for sevoflurane. It is obvious that there is no consensus on the occupational exposure limits for anesthetic gases and this is probably due to the point that enough information does not exist about the effect of waste anesthetic gases on humans and the results of previous investigations have been conflicting. Thus, there is a need for studies, in which the trace concentrations of waste anesthetic gases in the operating room and their effects on exposed personnel are simultaneously evaluated. It is not easy to reveal a causal relationship, so larger observational trials can help find it out, as well.

The present study, therefore, is aimed to measure the trace concentrations of halogenated anesthetic agents in the breathing zone air of personnel and then evaluate the possible adverse effects on hepatic and renal functions as well as oxidative stress parameters. Another goal of this study was to examine whether having longer working experience might have an effect on the adverse effects of anesthetic gases.

Methods

Chemical

All the chemicals were obtained from Sigma-Aldrich (GmbH, Munich, Germany) unless otherwise mentioned. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinine kits were purchased from Pars Azmoon Inc. (Tehran, Iran). Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) ELISA kits were obtained from Zell Bio GmbH Co. (Ulm, Germany).

Table 1. Characteristics of the study population.

Variables	Non-exposed personnel	Exposed personnel	*p* value
Number of subjects	30	42	
Male/female	13/17	18/24	
Working life (years; mean ± SD)	9.96 ± 8.12	11.25 ± 6.81	0.480
Age (years; mean ± SD)	32.41 ± 11.04	30.18 ± 9.22	0.355
Body mass index	27.21 ± 5.31	25.28 ± 3.38	0.0638

Study design

The present study was approved by the Scientific and Ethical Review Board of Urmia University of Medical Sciences on 29 September 2017 (IR.UMSU.REC. 1396–09-70–2870). This cross-sectional study was conducted on the hospital personnel of Urmia University of Medical Sciences, Urmia, Iran. We enrolled 42 OR personnel who were exposed to inhalational anesthetic gases and 30 healthy hospital personnel who were not exposed to these gases in any period of their life as the control group. The exposed personnel was assigned to the tasks of anesthesia technician, OR technician and surgical nurses. The anesthetic gases mainly used in the ORs of our study were: isoflurane and sevoflurane mixed with N_2O and oxygen. OR personnel were sub-divided into two groups: (a) those with more than 10 years of work experience ($n = 11$) and (b) those with less than 10 years of work experience ($n = 31$). The non-exposed group consisted of nurses, attending physicians and specialists working in the same hospitals. Other characteristics of the participants are summarized in Table 1. Our inclusion criteria were that the staff had worked at least 1 year (6 h daily) in the ORs and other parts of the hospital. All the exposed and unexposed personnel were asymptomatic with an unremarkable medical history and a normal physical examination. Individuals were excluded if they had undergone any surgical operations within the previous 3 months or had conditions that could influence the levels of oxidative stress parameters, kidney and liver indexes such as autoimmune diseases, liver or renal disease and acute/chronic inflammation. Moreover, those who were smoking or drinking alcohol or those taking any medications (e.g. vitamin supplements and antioxidants) were not included in

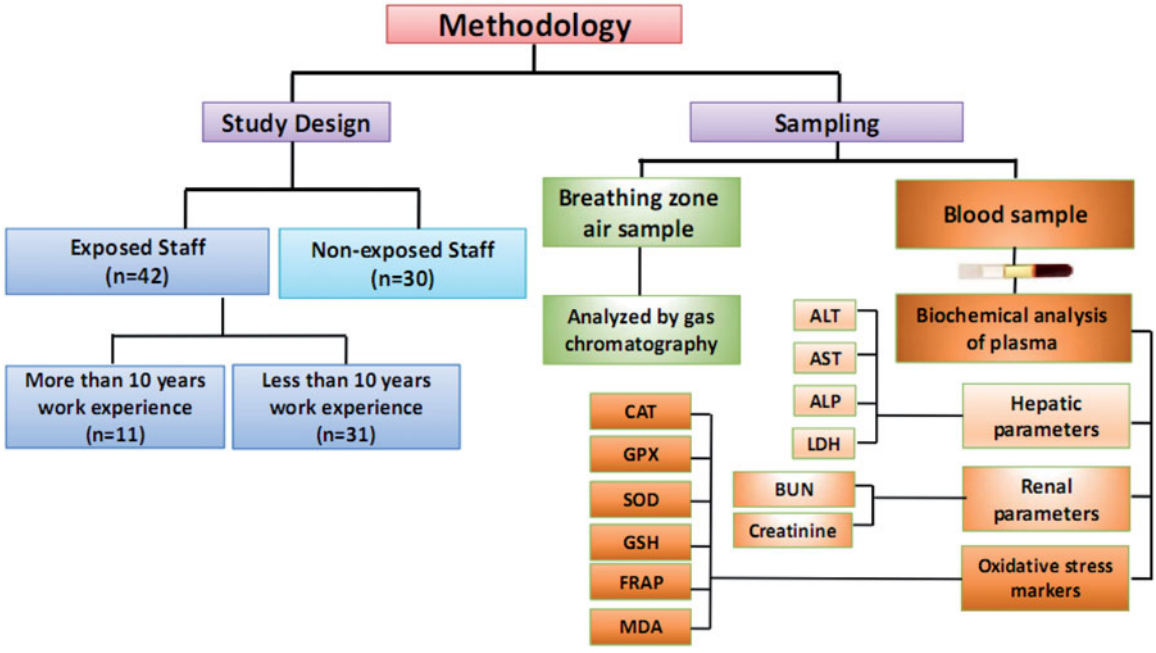

Figure 1. The methodology of the present study is illustrated. ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; BUN: blood-urea nitrogen; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde; GSH: reduced glutathione; FRAP: ferric reducing antioxidant power.

the present study. The present study was carried out in two parts. In the first part, the concentration of halogenated anesthetic gases was measured in the breathing zone air of the personnel during the shift work. In the second part, the fasting blood samples were taken into heparinized tubes from the same personnel in the next morning. All the blood samples were immediately transferred to the laboratory on ice and centrifuged for 10 min at 3000 rpm and 4 °C. Plasma was then separated and stored at −80 °C until analysis. The flowchart of the study design is shown in Figure 1. It should be noted that, among the halogenated agents, only isoflurane and sevoflurane were used in ORs of Urmia University of Medical Sciences. In general, anesthesia was induced using the intravenous anesthetics (such as propofol, thiopental, etomidate, ketamine, etc.) and maintained using the inhalational anesthetics (such as isoflurane, sevoflurane, and N_2O). The concentration of the used anesthetic drugs depends on the type of surgery, patient (child or adults), and so on. In the present study, the concentration of halogenated agents was measured in the air of several types of OR, including ENT, orthopedic, neurosurgery, urology and general, and all of them had the scavenging and ventilation systems (with relatively high ventilation rates of 15 times per hour).

Air monitoring

Isoflurane and sevoflurane were measured in the breathing zone of the personnel in accordance with OSHA 103 method (OSHA, 1994). In brief, air samplings were performed by the adsorbent tube (Anasorb 747, SKC, PA, USA) and low-flow pumps (Pocket Pump 210–1002TX, SKC, PA, USA) at 50 ml/min. Air sampling was carried out during the whole shift of a working day. To evaluate the precise exposure of the personnel, the samples were collected by active sorbent tube attached to clothing within the breathing zone of the personnel exposed to halogenated anesthetic agents; then, the adsorbent tubes containing the analytes were transferred to the laboratory. Finally, isoflurane and sevoflurane were extracted by 1 ml carbon disulfide from the adsorbent tube and analyzed using gas chromatography/flame ionization detector (GC-FID) and a capillary column (30 m × 0.32 mm ID × 0.25 μmdf). The temperature program was as follows: the initial oven temperature of 40 °C (held for 3 min) and, then, a 20 °C/min ramp to 100 °C (held for 10 min). The temperatures for the injector and detector were set at 200 °C and 280 °C, respectively. The flow rate of the carrier gas (nitrogen) was 0.5 ml/min. It should be noted that limit of detection (LOD) of isoflurane and sevoflurane were 0.01 and 0.008 ppm, respectively. More technical details have

been described in our previous paper (Jafari et al. 2018).

Biochemical analysis of blood samples

The plasma levels of ALT, AST, ALP, LDH, BUN, and creatinine were estimated using commercial reagent kits in accordance with the manufacturer's instruction. Method sensitivities for ALT, AST, ALP, and LDH were 4, 2, 3, and 5 U/L, respectively. The detection limits of BUN and creatinine were 2 and 0.1 mg/dl, respectively. The normal range value for ALT was lower than 31 U/L in females and lower than 41 U/L in males; for AST, lower than 31 U/L in females and lower than 37 U/L in males; and for ALP, 64–306 U/l in females and 80–306 U/l in males; for LDH, lower than 480 U/L both in females and males; for BUN, 8–44 mg/dl both in females and males; for creatinine, 0.6–1.3 mg/dl in females and 0.7–1.4 U/l in males. The activity of CAT, SOD, and GPx was determined by Elisa kits as described by the manufacturer. Method sensitivities for CAT, SOD, and GPx were 0.5, 1, and 5 U/ml, respectively. The assay range for CAT, SOD and GPx was 1–00, 5–100 and 20–500 U/ml, respectively.

The malondialdehyde (MDA) levels of the blood samples were determined by thiobarbituric acid (TBA) method. Briefly, an aliquot (200 µl) of plasma was mixed and shaken with 20% trichloroacetic acid (1 ml). Then, 150 µl of TBA (0.67% w/v) was added to the mixture, kept in boiling water bath (95 °C) for 30 min, followed by rapid cooling. Afterward, TBA-MDA adducts were extracted with n-butanol by vigorous shaking. The mixture was centrifuged at 3000 rpm for 10 min and the n-butanol layer was transferred to a separate tube. The absorbance of TBA-MDA adducts was recorded at 532 nm. The method was calibrated by tetraethoxypropane, as standard solutions (Kei 1978).

The plasma total antioxidant power was determined by the FRAP (ferric reducing antioxidant power) method. This method is based on the ability of plasma to reduce Fe (III) to Fe (II) in the presence of TPTZ (2,4,6-Tri(2- pyridyl)-s-triazine), forming a blue color $Fe2^{+}$–TPTZ complex with the absorption maximum at 593 nm. As described previously (Benzie and Strain 1996), ethanolic solutions of the known FeSO4 concentration were used as standard solutions to obtain the calibration curve. Fe (II) (1 µmol/ml) is equivalent to 1 µmol/ml of FRAP.

The reduced glutathione (GSH) levels of plasma sampleswere determined as described previously (Hu 1994). In brief, a volume of plasma (10 µl) was mixed with 200 µl of Tris–EDTA buffer (Tris base [0.25M], EDTA [20 mM], pH 8.2), followed by the addition of 4 µl of 10 mM of DTNB in methanol. Then the mixture was incubated at 37 °C for 30 min and the resulting yellow color was measured at 412 nm. Total GSH data were expressed as µmol/ml.

Table 2. Isoflurane concentration (ppm) in breathing zone of operating room (OR) personnel.

Job title	Number of individual	Median	Range
Anesthesia technician	20	2.21	(0.24–14.98)
OR technician	15	2.18	(0.38–5.31)
Surgical nurse	7	0.93	(0.19–2.36)
Total	42	1.33	(0.19–14.98)
p value	0.201		

Statistical analysis

All the statistical analyses were carried out using SPSS 16. Parametric data were analyzed using the Student's two-tailed *t*-test for unpaired findings. The Mann-Whitney test was used to analyze non-normally distributed data. To compare the isoflurane concentrations among the job titles, the nonparametric Kruskal-Wallis test was used. The differences between the frequencies of the variables (ALT, AST, ALP, LDH, BUN, and creatinine) were compared using Chi-square test. The *p* values of less than 0.05 indicated a significant difference.

Results

As illustrated in Table 1, there were no significant differences between the exposed and non-exposed groups with respect to working life, age, weight, and body mass index ($p > 0.05$). The sevoflurane concentration in the breathing zone of OR personal was less than LOD (0.008 ppm) and, hence, sevoflurane results are not indicated in Table 2. But isoflurane levels in the breathing zone of all OR personal were above LOD. Based on our findings, 52.38% of OR personnel were exposed to the isoflurane more than 2 ppm. The maximum and minimum isoflurane concentrations were found in the breathing zone air of anesthesia technicians and surgical nurses, respectively (Table 2). However, there was no significant difference among the job titles in term of isoflurane concentrations ($p > 0.05$). Also, the concentration of isoflurane and sevoflurane was measured in the breathing zone of the hospital personnel who had not worked in the ORs and results showed that these subjects were not exposed to these anesthetic gases (data not shown)

Table 3. Hepatic and renal parameters in exposed and non-exposed personnel.

Variables	Non-exposed personnel ($n = 30$)	Exposed personnel ($n = 42$)	p value
ALT (U/L) mean ± SD (range)	24.47 ± 7.83 (11.02–49.17)	32.17 ± 12.64 (10.19–71.32)	0.004
AST (U/L) mean ± SD (range)	21.93 ± 8.52 (10.17–51.84)	28.67 ± 10.34 (8.21–59.55)	0.005
ALP (U/L) mean ± SD (range)	189.24 ± 38.53 (113.67–261.34)	202.74 ± 56.62 (109.26–297.67)	0.262
LDH (U/L) mean ± SD (range)	311.61 ± 53.13 (178.33–497.64)	376.42 ± 81.92 (202.13–587.31)	0.001
BUN (mg/dl) mean ± SD (range)	11.37 ± 4.81 (8.13–17.52)	12.94 ± 3.59 (7.79–27.61)	0.118
Creatinine (mg/dl) mean ± SD (range)	0.94 ± 0.22 (0.73–1.32)	0.92 ± 0.14 (0.71–1.24)	0.698

ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; BUN: blood-urea nitrogen.

Table 4. Prevalence of individual kidney and liver blood markers above the normal range of laboratory.

Variables	Non-exposed personnel ($n = 30$)	Exposed personnel ($n = 42$)	p value
ALT	1 (3.33%)	8 (19.05%)	0.046
AST	1 (3.33%)	8 (19.05%)	0.046
ALP	0 (0%)	0 (0%)	–
LDH	1 (3.33%)	2 (4.76%)	0.764
BUN	0 (0%)	1 (2.38%)	–
Creatinine	1 (3.33%)	0 (0%)	–

ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; BUN: blood-urea nitrogen.

Table 5. Oxidative stress parameters in in exposed and non-exposed personnel.

Variables	Non-exposed personnel ($n = 30$)	Exposed personnel ($n = 42$)	p value
CAT (U/ml)	11.94 ± 2.33	7.16 ± 3.81	0.000
SOD (U/ml)	16.31 ± 0.27	12.87 ± 0.49	0.000
GPx (U/ml)	192.74 ± 18.12	136.39 ± 31.06	0.000
MDA content (nmol/ml)	1.62 ± 0.19	3.74 ± 0.41	0.000
GSH (μmol/ml)	0.331 ± 0.04	0.186 ± 0.10	0.000
FRAP (μmol/ml)	2.21 ± 0.57	1.88 ± 0.89	0.0787

CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde; GSH: reduced glutathione; FRAP: ferric reducing antioxidant power.

and no peak was observed in the position of isoflurane and sevoflurane in gas chromatogram.

The levels of ALT, AST and LDH in the exposed group were significantly higher than the control or non-exposed group ($p < 0.05$, Table 3). No significant difference was found between the exposed and non-exposed group in terms of plasma BUN and creatinine levels ($p > 0.05$, Tables 3 and 4). The Chi-square test indicated a statistically significant prevalence of the exposed personnel with an increase above the normal range of ALT and AST levels compared to unexposed controls (Table 4). It should be noted that no significant difference was found between males and females based on the normal ranges ($p > 0.05$).

The activity of CAT, SOD and GPx enzymes in the plasma samples of OR personnel were significantly lower than the personnel who were not exposed to anesthetic gases ($p < 0.05$, Table 5). A statistically significant increase was observed in the plasma MDA levels of exposed group in comparison to the control group (Table 5). The GSH levels were significantly lower in the plasma sample of OR personnel ($p < 0.05$, Table 5); however, no significant difference was found between the exposed and control groups in terms of FRAP value ($p > 0.05$).

As shown in Figure 2, the plasma levels of ALT and AST in OR personnel with more than 10 years of work experience were significantly higher than those of the personnel who had no more than 10 years of work experience ($p < 0.05$). The OR personnel with more than 10 years of work experience had significantly lower levels of GSH and antioxidant enzymes (CAT, GPx, and SOD) activities in comparison to those with less than 10 years of work experience and significant increment was found in the plasma MDA levels of the group with more than 10 years of work experience ($p < 0.05$, Figure 3). There was no significant difference between the OR personnel with more and less than 10 years of work experience in terms of other biochemical parameters used in this study ($p > 0.05$).

Discussion

The possible adverse effects of long-term exposure to trace levels of anesthetic gases in OR personnel has been a matter of concern (Irwin *et al.* 2009, Smith 2010). In the present study, it was found that chronic exposure to halogenated anesthetics, even at low levels, can result in a significant increase of liver markers and lipid peroxidation, which was probably due to the induction of oxidative stress by anesthetic agents.

A large number of studies have been conducted on ORs to measure the trace concentration of inhalational anesthetic agents in order to evaluate the efficiency of control equipment and instruments (e.g. scavenging systems and ventilators; Hallonsten 1982, Hoerauf *et al.* 1996). In this study, the concentration of isoflurane and sevoflurane in the breathing zone air of OR staff was measured by GC-FID. The obtained results indicated that the occupational exposure of OR staff to these halogenated agents was lower than national recommended exposure limits (REL). It meant that the scavenging and ventilation systems were efficient in

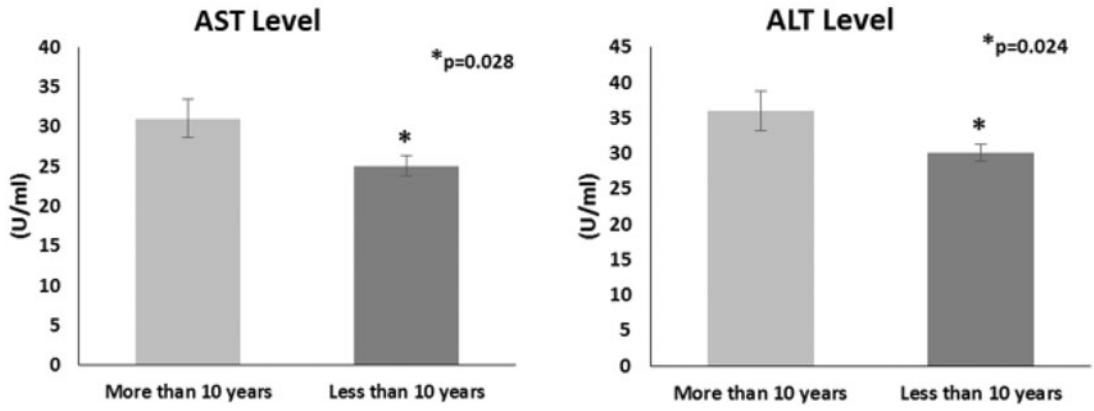

Figure 2. The activity of alanine transaminase (ALT) and aspartate transaminase (AST) in plasma sample of personnel with more (vs. less) than 10 years of work experience. Values are presented as mean ± SEM.

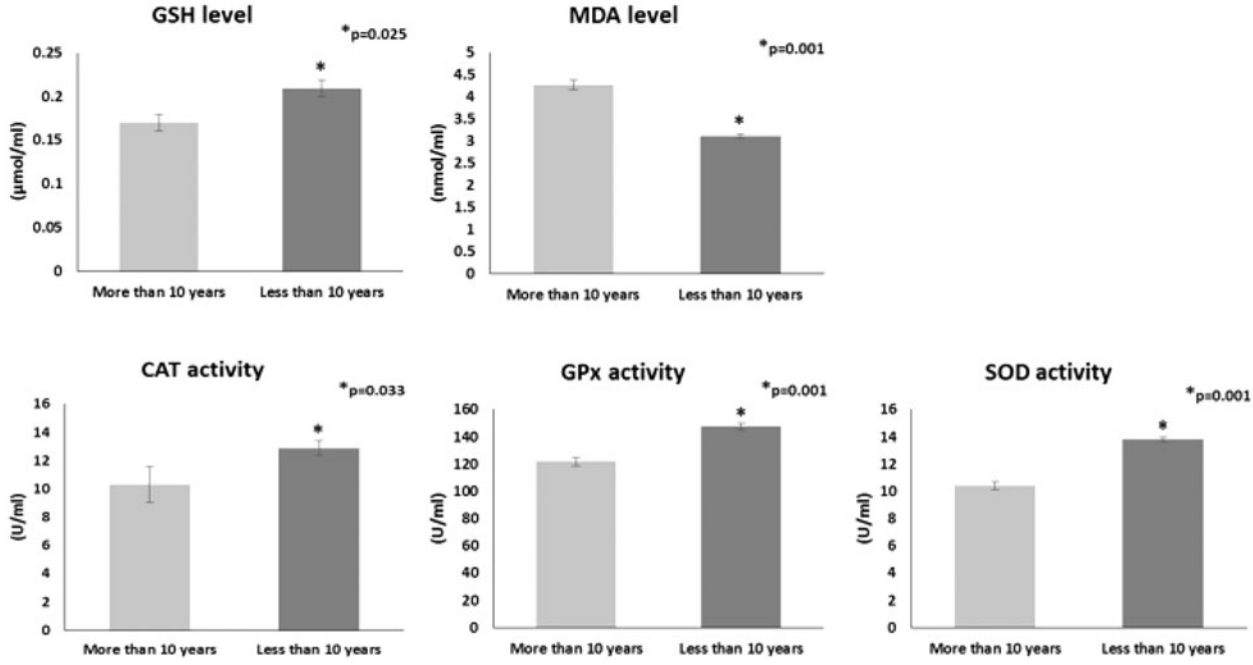

Figure 3. Oxidative stress parameters in plasma sample of personnel with more (vs. less) than 10 years of work experience. Values are presented as mean ± SEM. CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde; GSH: reduced glutathione.

the removal of the waste anesthetic gases from the ambient air of the work environment according to Iranian regulations. The sevoflurane concentration in the personal breathing zone was less than the detectable level (0.008 ppm). Although the MAC of sevoflurane is higher than isoflurane and thus the sevoflurane pollution in the air of the operating rooms is expected to be higher than isoflurane, the results of our study demonstrated the opposite. This was because of the cost of sevoflurane. Due to its high price, sevoflurane is rarely used in comparison to other halogenated anesthetics in Iran. According to the questionnaire's information, unfortunately, sevoflurane was not used when we collected our samples from the air of the operating rooms; that is why sevoflurane was not detected in this study. The levels of isoflurane found in the present study exceeded the NIOSH REL in most subjects. Some researchers believe that the NIOSH limits are very conservative and quite strict compared to those of other countries (Hoerauf *et al.* 1996; 1999, Byhahn *et al.* 2001). Thus, one of the main goals of our study was to know whether chronic exposure to anesthetic gases at the concentrations above the NIOSH limit would really cause undesirable health effects. The range of isoflurane concentration in the air of the operating rooms was very large, which can

be attributed to the type of surgery, flow gas, efficiency of scavenging and ventilation systems, connections of anesthetic machines, induction techniques, skill level of personnel, etc.

There are some studies in the literature that have examined the concentrations of waste anesthetic gases as well as several biological effects on the exposed personnel. For instance, Souza et al. evaluated DNA damage, genomic instability, cell death and proliferative index in exfoliated buccal cells from anesthesiologists and determined the concentrations of the anesthetic gases most commonly used in ORs. They found that although the exposure to trace levels of waste anesthetic gases did not induce DNA damage, it can lead to genomic instability, cytotoxicity and proliferative changes in the exposed personnel (Souza *et al.* 2016). In another study by Wronska-Nofer *et al.* the extent of oxidative DNA damage in relation to the level of exposure to N_2O and halogenated hydrocarbons was investigated in 36 female nurses. Their results showed the positive correlation between the oxidative DNA damage and the N_2O levels in the OR air, whereas no association was found between genotoxic effects and sevoflurane or isoflurane (Wronska-Nofer *et al.* 2012). This observation was not consistent with our findings. In our study, the concentration of halogenated anesthetics in the OR air and their biological effects (through liver, kidney, and oxidative stress parameters) on the operating room personnel were investigated. Although the concentration of N_2O was not measured in the current study, our results demonstrated that the long-term exposure to anesthetic agents could lead to an increase in the activities of transaminase enzymes (ALT and AST), but not ALP, in the exposed personnel. This is probably due to the differences in the concentration of halogenated anesthetic gases. The isoflurane concentrations in our study ranged between 0.19 and 14.98 ppm and 52.38% of them was more than NIOSH REL (2 ppm). However, in the study by Wronska-Nofer *et al.*, the range of isoflurane and sevoflurane concentration were 0.05–1.98 ppm (0.4–15.0 mg/m^3) and 0.04–1.71 ppm (0.5–14.0 mg/m^3), respectively, which was below NIOSH REL (Wronska-Nofer *et al.* 2012). It seems that the adverse health effects of halogenated anesthetic gases are dependent on the magnitude of exposure. Our findings were consistent with the report of Casale *et al.*, in which the effect of occupational exposure to low dose of anesthetic gases in blood parameters in health care workers was investigated and the results showed that the blood levels of hepatic parameter such as AST, ALT, GGT, and total bilirubin were significantly increased in the exposed workers in comparison to the non-exposed ones (Casale *et al.* 2014). Toprak *et al.* and Nishiyama have reported that the subjects exposed to isoflurane had a significant increase in hepatic transaminases activities (Toprak *et al.* 2012, Nishiyama 2013). Moreover, a recently published paper showed no significant difference between the unexposed and exposed personnel (those who were exposed to sub-TLV levels of halothane) in terms of AST and ALT, but when the two groups were compared with regard to gender, a significant difference was observed between the exposed females and control group with regard to the mean of AST and ALT (Bakhshaei *et al.* 2017).

The plasma creatinine and BUN levels have been used to assess the possible harmful effects of chronic exposure to low anesthetic concentrations on the renal function. The results of this study demonstrated no statistically significant difference between the exposed and non-exposed groups in terms of creatinine and BUN levels. According to previous works, methoxyflurane and sevoflurane are biodegraded to some metabolites (e.g. inorganic fluoride and compound A) that can induce nephrotoxicity and even lead to renal failure (Eger *et al.* 1997; McGregor 2000, Kharasch *et al.* 2001). Most of the human studies regarding the effects of halogenated anesthetic agents on the kidney function have been done on patients, not OR personnel. Only in a study by Trevisan *et al.*, the effects of N_2O and sevoflurane were evaluated on the kidney of the exposed subjects and the results of their study showed that the renal biomarkers were not affected by these anesthetic agents and remained within the normal range (Trevisan *et al.* 2003). Other investigations, which have been performed on the patients in this context, are in agreement with our results. Higuchi *et al.* investigated the renal effect of prolonged low-flow sevoflurane and isoflurane anesthesia in surgical patients with no preexisting renal disease and found no significant changes in the BUN, serum creatinine concentrations and creatinine clearance in all the patients after anesthesia (Higuchi *et al.* 1998). Reichle et al reported that the administration of modern halogenated anesthetics had no significant adverse effect on the patients' renal function and even patients with stable renal insufficiency could be anesthetized with sevoflurane as safely as any other halogenated anesthetic agent (Reichle *et al.* 2002).

In this study, oxidative stress status was also assessed in OR staff chronically exposed to waste anesthetic gases. As previously reported in several studies, oxidative stress is a potential mechanism of

toxicity of many chemicals (such as metals, anesthetic agents, etc.; Ghaffarian-Bahraman *et al.* 2014, Cegin *et al.* 2016). Oxidative damage is usually initiated by the generation of reactive oxygen radicals (e.g. superoxide, hydroxyl radical, and peroxides). In normal condition, superoxide radicals have a short life and are rapidly converted into hydrogen peroxide. Peroxides, hydroxyl and other reactive radicals are eliminated by enzymatic (such as CAT, SOD, and GPx) and non-enzymatic (such as GSH) antioxidants. Otherwise, these free radicals can attack cellular macromolecules, especially membrane lipids and induce lipid peroxidation (Baghaei *et al.* 2016). The result of the present study indicated that long-term exposure to the waste of anesthetic gases can induce oxidative stress as manifested by the rise in transaminase activities accompanied by the marked decline of enzymatic and non-enzymatic antioxidant levels as well as the rise of MDA levels. These results were in agreement with the literature data. In a study by Malekirad *et al.*, the status of oxidative stress in OR personnel was assessed by measuring the FRAP, GSH, and MDA levels in plasma samples. Their results showed that the GSH and MDA levels in the workers exposed to volatile anesthetics were significantly different from those of the unexposed workers. They suggested that administration of safe antioxidants (i.e. vitamins C and E as well as selenium) might protect OR staff from adverse health effects of inhalational anesthetic agents (Malekirad *et al.* 2005). Ranjbar *et al.* conducted a clinical trial on the OR personnel to examine the anti-oxidative potential of cinnamon and found that lipid peroxidation in OR workers decreased following the use of cinnamon tea (Ranjbar *et al.* 2007). In Turkey, Turkan *et al.* and Cegin *et al.* have investigated the effect of occupational exposure to volatile anesthetics on oxidative stress. Their findings indicated that the levels of CAT activity, sulfhydryl group and trace element were meaningfully lower in OR staff compared to that of in the control groups, while lipid hydroperoxide concentration and myeloperoxidase activity were significantly higher (Türkan *et al.* 2005, Cegin *et al.* 2016). Costa Paes et al reported that occupational exposure to waste anesthetic gases not only changes in redox status during medical residency, but also can increase DNA damage (Costa Paes *et al.* 2014).

The transaminase levels in OR personnel with more than 10 years of work experience were higher than those of personnel who had less than 10 years of work experience. These results were confirmed by oxidative stress parameters. The levels of antioxidant enzymes and lipid peroxidation in OR personnel with more than 10 years of work experience were significantly different from those of personnel with less than 10 years of work experience. It seems that these differences had two main reasons: first, duration of exposure; second, exposure to different anesthetic agents. It was obvious that OR personnel with more years of work experience had longer exposure to waste anesthetic gases, and our findings showed that longer exposure to anesthetic agents may result in more adverse health effects, even at low levels. In the analysis of the questionnaire, it was realized that the OR personnel with more than 10 years of work experience were previously exposed to halothane that is no longer used. Up to about 15 years ago, halothane was routinely used in ORs of Urmia University of Medical Sciences.

According to the questionnaire, all the OR personnel were previously exposed to N_2O and sevoflurane; hence, the results of biochemical assays performed on blood samples cannot be attributed solely to the concentration of isoflurane. Considering that the volatile anesthetics were usually used in combination, it is not possible to blame one anesthetic agent for the hepatic or oxidative damage (Türkan *et al.* 2005). This was a limitation of our study and all other investigations which were conducted in ORs in this context. Overall, it is very hard to investigate the long-term effects of a particular anesthetic gas on occupationally exposed personnel since they are usually used as a mixture of several anesthetic agents.

Since possible health hazards from chronic exposure to volatile anesthetics cannot yet be definitively excluded, the US-NIOSH recommends 25 ppm for N_2O and 2 ppm for halogenated agents (Jafari *et al.* 2018). Seemingly, the NIOSH REL not only is not strict and conservative, but also it is better to be used in other countries because it seems that the NIOSH REL can effectively guarantee the health of the staff exposed to inhalational anesthetic agents. Although the maximum isoflurane concentration found in this study was less than 15 ppm, which is much less than the national REL (50 ppm), some of the OR personnel exposed to waste anesthetic agents have hepatic and oxidative damage according to biochemical parameters and the occupational exposure limit for halogenated gases in Iran should decrease to 2 ppm (Hajaghazadeh and Jafari 2018). It is clear that reducing the occupational exposure limits alone is not enough and some measures should be taken to reduce the concentrations of waste anesthetic gases in the ORs below the NIOSH limits.

In conclusion, the occupational exposure of the OR personnel to isoflurane and sevoflurane was less than the national REL (50 ppm); however, the levels of isoflurane exceeded the NIOSH REL in the breathing zone of some OR personnel. Biochemical analysis of plasma samples showed that chronic exposure to trace levels of anesthetic gases can result in oxidative and hepatic damage in some OR personnel; these adverse effects increase with increasing work experience. Although exposure to waste anesthetic gases in ORs is unavoidable, their adverse health effects can be minimized by taking some measures, such as using effective ventilation and scavenging systems as well as checking regularly all the anesthetic equipment in order to reduce OR pollution with anesthetic gases.

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