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# Oxidative stress in operating room personnel: occupational exposure to anesthetic gases

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Health professionals exposed to anesthetic gases are at higher risk of reproductive, neurological, hematological, immunological, hepatic and renal system diseases. We investigated if oxidative stress induced by chronic exposure to anesthetic gases has any association with this matter. Plasma lipid peroxidation, total antioxidant capacity and total thiol molecule levels were measured in 66 operating room staff in comparison with 66 controls. The exposed group had a significantly higher level of lipid peroxidation with decreased thiol groups compared to control subjects. Total antioxidant capacity of the body was no different among exposed and not exposed subjects.

Increased lipid peroxidation in the blood of exposed subjects warns that oxygen free radicals have increased in

the body and thus might attack cells, which, in the long-term, results in multi-organ damage. The remaining blood total antioxidant capacity at normal values is promising and means that other non-thiol antioxidants, such as uric acid, transferrin, ceruloplasmin, albumin, and vitamin antioxidants, such as  $\alpha$ -tocopherol and ascorbic acid, have been stimulated to maintain the total anti-oxidant power of the body at normal state. *Human & Experimental Toxicology* (2005) 24, 597–601

**Key words:** lipid peroxidation; operating room personnel; oxidative stress; toxicity

## Introduction

Oxidative stress arises when there is a marked imbalance between the production and removal of reactive oxygen species (ROS). In contrast to acute oxidative stress, chronic existence of excessive amounts of free radicals may lead to several irreversible effects, such as fibrosis, necrosis, atrophy, vascular damage and DNA breakage.<sup>1</sup>

Anesthetic gases are one group of agents capable of inducing oxidative stress. Although several efforts have been made to minimize the exposure to waste anesthetic gases in operating rooms – improving the working environment – operating room air contamination is still unavoidable.<sup>2,3</sup> The

potential detrimental chronic effects of anesthetic gases on reproductive, neurological, hematological, immunological, hepatic and renal systems, plus the possibilities of increased cancer risk have been the subject of previous research.<sup>4,5</sup> However, the pathophysiology of these adverse effects of anesthetic gases is still unknown. One hypothesis could include the induction of oxidative stress by these gases in vital organs. Chronic exposure to ROS may explain a causal biologic pathway, which leads to the aforementioned illnesses.

To shed more light on this theory and also to elucidate the status of oxidative stress and antioxidant capacity in operating room personnel, we investigated their antioxidant status compared to a hospital-based control group using the ferric reducing ability of plasma (FRAP) method and measured total thiol groups and plasma thiobarbituric acid reactive substances (TBARS) as markers of lipid peroxidation.

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## Methods

### Subjects

We designed a comparative cross-sectional study with 132 subjects. The first cohort included 66 full-time workers in operating rooms at two Arak State Hospitals in Arak, Iran. None of these workers were exposed in the workplace to any hazardous agents other than anesthetic gases. The control group comprised of 66 individuals working in the same hospitals without any work-related exposure to hazardous agents (e.g., radiation and anesthetic gases). All subjects in both groups lived in the same urban area. The study was conducted in accordance with the declaration of Helsinki and all participants were provided with specific written information on the aims of the study before written consent was obtained. Prior to blood collection, each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire specifying gender, date of birth, smoking status, dietary habits, work-related exposure to hazardous agents, previous exposure to diagnostic X-ray as a patient, recent local or general operation with anesthesia, consumption of vitamin supplements and other antioxidants, and use of therapeutic drugs. No study subject consumed alcohol as it is prohibited in the country.

Subjects who had a history of surgery under anesthesia in the previous year or received diagnostic or therapeutic X-ray exposure (as confounders for oxidative stress) or chemotherapeutic drugs were excluded. Vegetarians and those who used vitamin supplements, antioxidants or any therapeutic drugs were also excluded.

Both hospital operating rooms, equipped with air conditioning facilities but no active scavenging devices, are used for general surgical purposes. Considering anesthesia techniques in adults, anesthesia was induced intravenously and maintained with inhaled anesthetics supplemented by opioids. Tracheal tubes with blocked cuffs were used. The fresh gas flow was approximately 4 L/min. Halothane and N<sub>2</sub>O were the most frequently used inhaled anesthetics in these settings.

### Chemicals

Dithionitrobenzoic acid (DTNB), Tris base, 1,1,3,3'-tetraethoxypropane (Sigma, UK), 2-thiobarbituric acid (TBA), *n*-butanol (Merck, Germany), 2,4,6-tripyridyl-*s*-triazine (TPTZ) (Fluka, Italy) were used in this study.

### Sampling and laboratory measurements

All blood samples were drawn by venipuncture and coded in the occupational medicine unit. The processing and scoring of the samples of the two groups were then performed blinded and concurrently in the laboratory. At the end of the study, the data from the questionnaire and the radiation burden records were linked to the code number for data analysis.

The FRAP method was used for plasma total antioxidant measurement. This method was formed based on the ability of plasma to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ. The absorbance of the Fe<sup>2+</sup>-TPTZ complex formed was measured at 593 nm using a spectrophotometer.<sup>6</sup>

The TBA method was used to measure the rate of lipid peroxidation. In this method, plasma samples were mixed with TCA (20%) and the precipitate was dispersed in H<sub>2</sub>SO<sub>4</sub> (0.05 M). TBA (0.2% in sodium sulfate 2 M) was added and heated for 30 min in a boiling water bath. TBARS adducts were extracted by *n*-butanol and the absorbance was measured at 532 nm in a spectrophotometer.<sup>7</sup>

DTNB was used to evaluate the plasma thiol groups. DTNB reacted with the thiol groups to yield a yellow complex with absorbance at 412 nm in spectrophotometer.<sup>8</sup>

### Statistical analysis

A detailed multi-variable database was formed. All data were collected either as dichotomous variables (e.g., sex, exposure) or as continuous variables (e.g., laboratory measurements). All data were analysed with STATA software, version 8.2. The two sample *t*-test was used for statistical comparisons after plotting and testing for equal variances. Simple multivariate regression was used to test for a linear correlation between age and employment duration as well as other variables. Type I error was chosen at a level of 5%.

## Results

Basic characteristics of the subjects are shown in Table 1. There was no statistically significant differences between the two groups in terms of age, gender, smoking, and years of employment.

The levels (mean ± SE) of TBARS, SH groups and FRAP are presented in Table 2. A significant increase in lipid peroxidation concentration (*P* < 0.01) was observed in the blood of operating room personnel measured as TBARS concentration. The values for operating room personnel and

**Table 1** Basic characteristics of the study sample

Group	Mean age	Sex (M/F)	Mean smokers (%)	Mean employment years
Exposed personnel (n = 66)	32	46/20	2	9.34
Non-exposed personnel (n = 66)	32	46/20	2	9.68

controls were  $13.12 \pm 0.5$  and  $10.96 \pm 0.42$  nmol/mL, respectively.

The mean FRAP concentration was not significantly different between the two groups ( $1.72 \pm 0.36$  versus  $1.87 \pm 0.54$   $\mu\text{mol/mL}$ ). Total SH groups of operating room personnel were significantly ( $P < 0.01$ ) lower than those of controls ( $0.17 \pm 0.008$  versus  $0.21 \pm 0.012$  mM).

## Discussion

In this study, oxidative stress status was investigated in operating room employees who were exposed to halothane and  $\text{NO}_2$ . Our results indicated that lipid peroxidation, as a marker of ROS concentration, was increased in operating room staff. In contrast, total thiol groups were markedly decreased in these subjects compared to the non-exposed group, but total antioxidant capacity remained unchanged. This may be explained by the fact that after chronic exposure to excessive amounts of oxygen radicals, the body's total thiol groups decrease, but in compensation, other types of antioxidants of the body are stimulated and thus total antioxidant capacity of the body remains normal.

The balance between the production of free radicals and the antioxidant defenses in the body has important health implications; if there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops which may cause chronic and permanent damage. The human body has several mechanisms to counteract the damage produced by free radicals; the basic and most prominent defense mechanism is antioxidant agents. The term antioxidant has been defined as any substance that delays or inhibits the oxidative damage to a target molecule. They are stable enough molecules that can neutralize free radicals by donating electrons. In the human body, antioxidants can be categorized in two main sys-

tems. The main system of defense against damage from free radicals is an enzymatic system, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), that oppose oxidation. Secondly, the body maintains pools of the antioxidant vitamins, such as vitamins E and C and the vitamin A precursor beta-carotene. The first defense system tries to handle all free radicals and if the oxidative stress is far greater than the capacity of the system, the second line of defense (vitamins) may come into action. Vitamins' activities scavenge and quench free radicals, becoming oxidized and inactivated in the process. Each of these antioxidant nutrients has specific activities and they often work synergistically to enhance the overall antioxidant capacity of the body.<sup>9</sup>

In a similar work recently published, anesthesia and surgery personnel who had been exposed to inhalation anesthetics for 3 years were compared to healthy volunteer personnel for blood SOD and GPx. Supporting the present findings on reduction of blood total thiol molecules, they reported that plasma and erythrocyte SOD and GPx activities and their co-factor levels were lower in operating room personnel.<sup>10</sup> However, they did not examine blood lipid peroxidation level. Therefore, the present study completes that work and reports for the first time that operating room personnel are tolerating increased and persistent oxidative stress. As found in the present study, the remaining blood total antioxidant capacity at normal values is very promising and means that other non-thiol antioxidants, including enzymatic and non-enzymatic, as described above, have been stimulated to maintain the total antioxidant capacity of the body at normal value. On the other hand, it is important to remember that lipid peroxidation is an inevitable accompaniment of cell death from any cause and increased TBARS found in the present study is hazardous. There is extensive evidence that oxidative stress is

**Table 2** Comparison of oxidative stress components among hospital personnel exposed or not exposed to anesthetic gases

Group	FRAP ( $\mu\text{mol/mL}$ )	TBARS (nmol/mL)	SH groups (mM)
Exposed personnel (n = 66)	$1.72 \pm 0.36$	$13.12 \pm 0.5^{**}$	$0.17 \pm 0.008^{**}$
Non-exposed personnel (n = 66)	$1.87 \pm 0.54$	$10.96 \pm 0.42$	$0.21 \pm 0.012$

Data are mean  $\pm$  SE. Total antioxidant capacity (FRAP), lipid peroxidation (TBARS), and thiol (SH) group concentrations were determined.

\*\*Represent the difference between two groups significant at  $P < 0.01$ .

**Table 3** Diseases in which oxidative stress is possibly involved in their pathophysiology

Disease category	Disease name
Autoimmune	Rheumatoid arthritis, immune-complex-mediated vasculitis, inflammatory bowel diseases
Eye	Cataract, age-related macular degeneration, retinopathy, cystic macular edema
GI tract	Hepatitis, pancreatitis, stomach, colitis
Kidney	Renal failure, renal interstitial fibrosis, nephropathy
Lung	Bronchial asthma, adult respiratory distress syndrome, cystic fibrosis, pneumonia, idiopathic pulmonary fibrosis, chronic obstructive pulmonary diseases
Neurodegenerative	Parkinson's, Huntington's, amyotrophic lateral sclerosis, progressive supranuclear palsy, Alzheimer's, multiple sclerosis, reflex sympathetic dystrophy, dementia, neuronal lipofuscinosis
Red cells	Sickle cell disease, anemia, aging, glucose-6-phosphate dehydrogenase activity, fetal/neonatal hypoxia, thalassemia, malaria infection
Skin	Contact dermatitis, atopic dermatitis, psoriasis, vitiligo
Vascular	Atherosclerosis, myocardial infarction, stroke, ischemic and reperfusion damage, focal cerebral ischemia, subarachnoid hemorrhage
Various	Trauma, cancer, burns, inflammatory conditions, multiple organ dysfunction, toxicity of xenobiotics

Adapted from Abdollahi *et al.* (2004).<sup>9</sup>

an important mechanism of many chronic diseases, such as neurodegeneration, Alzheimer, Parkinson, cataracts, atherosclerosis, neoplastic diseases, diabetes, chronic inflammatory diseases of the gastrointestinal tract, aging of skin, asthma etc., reviewed previously (Table 3).<sup>9</sup> This would be a reasonable and plausible etiology for numerous adverse health effects that have been reported for operating room personnel. Supporting the above-mentioned points, a meta-analysis of ten studies indicated an enhanced relative risks of hepatic disease (RR = 1.6), cervical cancer (RR = 1.4), liver disease (RR = 1.5) and kidney disease (RR = 1.3) in exposed males and females.<sup>11</sup> Another meta-analysis on 17 studies has shown an increased abortion rate among exposed females (RR = 1.48).<sup>12</sup>

Since possible health hazards from long-term exposure to inhalational anesthetics cannot yet be definitively excluded, many Western countries have established limits for exposure. These usually range from 2 to 10 ppm as a time-weighted average over the time of exposure. A number of investigations

have demonstrated that, in operating theatres with modern climate control and waste anaesthetic gas scavenging systems, occupational exposure is unlikely to exceed threshold limits. Occupational exposure from the use of volatile agents in operating theatres with poor air control, especially during bronchoscopy procedures in pediatric patients, remains a source of concern.<sup>13</sup>

For future investigations, we suggest using longitudinal, analytic studies to follow-up the staff of operating rooms with different air contamination status based on their air conditioning facilities or an experimental study concerning the effect of an antioxidant diet or therapy, to decipher the causal pathway between oxidative stress and increased risk of the aforementioned illnesses. Meanwhile, administration of proper doses of safe antioxidants (i.e., vitamins C and E and selenium) and installation of improved air ventilation facilities, such as active scavenging devices, seem reasonable for the protection of those who are chronically exposed to anesthetic gases.

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