

## Serum myeloperoxidase (MPO) activity, oxidative and antioxidative parameters in operating room personnel

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### Abstract

**Objectives:** To investigate the effects of occupational exposure to anaesthetic gases on myeloperoxidase activity, oxidative and antioxidative parameters in operating room personnel.

**Methods:** The cross-sectional study was conducted at Yuzuncu Yil University, Van, Turkey, in May 2011, and comprised equal number of operating room and non-operating room personnel. Serum myeloperoxidase activity, sulfhydryl group levels, lipid hydroperoxide levels and catalase activity were determined. SPSS 11 was used for data analysis.

**Results:** There were 64 subjects; 32(50%) each in the two groups. Myeloperoxidase activity and lipid hydroperoxide levels were significantly higher in operating room personnel than in the non-operating room personnel ( $p < 0.001$ ;  $p < 0.001$ ), while catalase activity and sulfhydryl group levels were significantly lower ( $p < 0.009$ ;  $p < 0.003$ ). Catalase activity negatively correlated with lipid hydroperoxide levels in operating room personnel ( $r = -0.293$ ;  $p = 0.018$ ). Myeloperoxidase activity negatively correlated with sulfhydryl group levels in operating room personnel ( $r = -0.267$ ;  $p = 0.031$ ).

**Conclusions:** Operating room personnel exhibited higher oxidative stress, which may be due to the oxidative effect of anaesthetic gases.

**Keywords:** Anaesthetic gases, Myeloperoxidase activity, Occupational exposure, Oxidative stress, Sulfhydryl groups. (JPMA 66: 666; 2016)

### Introduction

Anaesthetic gases are an important chemical hazard in the hospital environment due to their widespread use in operating theatres. Occupational exposure to anaesthetic gases may result in adverse health effects in operating room personnel.<sup>1</sup> In previous studies, the potentially detrimental chronic effects of anaesthetic gases such as halothane and enflurane on the reproductive, neurological, haematological, immunological, hepatic and renal systems were investigated.<sup>2</sup>

A limited number of studies have suggested that anaesthetic gases lead to oxidative injury, although the mechanism by which this occurs is largely unknown. The pathophysiology of the adverse effects of anaesthetic gases is still unknown. One hypothesis suggests that these gases induce oxidative stress (OS) in vital organs. It has been demonstrated that

several anaesthetic agents, such as desflurane, produce reactive oxygen species (ROS) and change serum antioxidant levels in operating room personnel.<sup>3</sup> Baysal et al.<sup>4</sup> investigated the effect of occupational exposure to anaesthetic gases on the oxidative status of operating room personnel. Turkan et al.<sup>5</sup> evaluated effect of volatile anaesthetics on OS due to occupational exposure. More recently, Lee et al.<sup>6</sup> reviewed impact of volatile anaesthetics, such as isoflurane and sevoflurane, on OS and inflammation.

Myeloperoxidase (MPO) is an oxidative enzyme present in neutrophils, monocytes and macrophages in the blood; there is a positive correlation between leukocyte count (LC) and MPO levels.<sup>7</sup> MPO exerts this effect by using chloride ions and cell-generated hydrogen peroxide to create hypochlorous acid (HOCl). The major end-product in plasma concentrations of chloride ions is generally thought to be HOCl. MPO is linked to OS. Additionally, MPO is a source of OS in the human artery wall.<sup>8</sup> MPO is a leukocyte-derived enzyme that generates potentially atherogenic ROS.<sup>9</sup> MPO has thus emerged as an important pro-atherogenic mediator in patients with advanced cardiovascular disease (CVD).<sup>10</sup>

Serum sulfhydryl groups of proteins constitute the main antioxidant component of serum. Blood contains many

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antioxidant molecules that prevent and/or inhibit harmful free radical reactions.<sup>11</sup> Catalase is a well-known intracellular antioxidant enzyme and is a member of the free radical and ROS scavenging system. Catalase, which detoxifies hydrogen peroxide, is an important enzyme in the antioxidant system, which defends against the OS that occurs in many pathological conditions, including cancer, diabetes, atherosclerosis, neuro-degenerative disease and aging.<sup>12</sup>

To our knowledge, serum MPO activity, catalase activity, sulfhydryl group levels, and OS among operating room personnel have not yet been reported. We hypothesised that serum MPO activity would be increased in operating room personnel due to the oxidative effects of anaesthetic agents.

The current study was planned to investigate the effects of occupational exposure to anaesthetic gases on MPO activity, oxidative and antioxidative parameters in operating room personnel.

### Subjects and Methods

The cross-sectional study was conducted at Yuzuncu Yil University, Van, Turkey, in May 2011, and comprised operating room personnel who volunteered to participate after permission had been obtained from the institutional ethics review committee.

We used a closed system for anaesthesiology equipment and passive scavenging systems for waste anaesthetic gas. The operating rooms also had air-conditioning and a central high-flow scavenging system.

Subjects included were operating room personnel having worked for a minimum of 6 hours daily for at least 3 years. They were asymptomatic, with an unremarkable medical history and a normal physical examination. Those who had undergone general anaesthesia within the preceding 3 months were excluded. Also excluded were those who had a history of alcohol abuse, smoking, intravenous (IV) drug abuse, pregnancy, antioxidant supplement use, active infection, hypertension, diabetes mellitus, hyperlipidaemia, liver or renal disease, rheumatoid arthritis (RA), coronary artery disease (CAD), or pulmonary disease.

The operating room personnel were exposed to a complex mixture of anaesthetic agents (sevoflurane, nitrous oxide and desflurane).

A medical history was obtained for all operating room and non-operating room personnel, with a specific focus on history of CAD. The subjects were then evaluated by standard physical examination, chest X-ray and baseline electrocardiography (ECG).

Blood samples were collected at rest between 0800 hours

and 0900 hours from an antecubital vein after overnight fasting. Blood samples were collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 1,409g for 10 minutes. Serum samples, which were used to measure MPO activity, catalase activity, sulfhydryl groups levels and lipid hydroperoxide (LOOH) concentration, were stored at -80°C until they were used.

Serum LOOH levels were measured with a ferrous ion oxidation-xylenol orange assay. The assay utilises the oxidation of ferrous ion to ferric ion via various oxidants; the production of ferric ion is then measured with xylenol orange. LOOHs are reduced by triphenyl phosphine (TPP), a specific reductant for lipids. The absorbance was measured at 560nm. The difference seen with and without TPP pre-treatment reflected LOOH levels.<sup>13</sup>

Catalase activity was measured using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a substrate.<sup>14</sup> The disappearance of H<sub>2</sub>O<sub>2</sub> was followed at 240nm. Catalase activity was expressed in units per litre of serum (U/L) at 25°C.

Serum MPO activity was determined utilising the method described by Klebanoff and Clark;<sup>15</sup> the formation rate of the yellowish-orange oxidation product of o-dianisidine and MPO in the presence of H<sub>2</sub>O<sub>2</sub> was measured at 460nm. One unit of MPO was defined as the quantity required to degrade 1 μmol of H<sub>2</sub>O<sub>2</sub> per minute at 25°C. The molar extinction coefficient of 1.3×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> of oxidised o-dianisidine was used for the calculation. MPO activity was expressed in units per litre of serum (U/L).

Free sulfhydryl groups of serum samples were assayed according to the method initially modified by Hu et al.<sup>11</sup> Briefly, 1 mL of buffer containing 0.1 M Tris, 10 mM Ethylenediaminetetraacetic acid (EDTA), pH 8.2, and 50 μL serum was added to cuvettes, followed by 50 μL of 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in methanol. Blanks were run for each sample as a test without DTNB. Following incubation for 15 minutes at room temperature, sample absorbance was read at 412nm on a Cecil 3000 spectrophotometer (Cecil Instruments, Cambridge, UK). The values of the sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was then calculated using reduced glutathione as the standard for free sulfhydryl groups, and the results were expressed in millimolars.

The levels of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were determined by using commercially available assay kits (Abbott®) with an autoanalyser (Aeroset®, Abbott®).

The data was analysed using SPSS 11. The results were expressed as the mean and standard deviation (SD). Prior to

analysis, all the groups' internal quality control was made. Parametric variables were compared using student's test. Qualitative data was compared using the  $\chi^2$  test. The correlation analyses were performed using Pearson's correlation test. The results were considered to be statistically significant when the p value was less than 0.05.

## Results

There were 32 operating personnel in the study; 17(53%) males and 15(47%) females. The other group had 32 non-operating room personnel; 18(56%) males, 14(44%) females. There were no significant differences between the groups with respect to age, body mass index (BMI) and duration of work in hospital ( $p>0.05$ ). Also, there were no significant differences between the groups with respect to serum TG, TC, LDL-C and HDL-C levels ( $p>0.05$ ) (Table-1).

Serum MPO activity and LOOH levels were significantly higher in operating room personnel than non-operating room personnel ( $p<0.001$ ;  $p<0.001$ ), while catalase activity and sulfhydryl group levels were significantly lower ( $p<0.009$ ;  $p<0.003$ ) (Table-2).

Serum catalase activity showed a significant correlation with LOOH levels in operating room personnel ( $r=-0.293$ ;  $p=0.018$ ). In addition, MPO activity significantly correlated

with sulfhydryl group levels in operating room personnel ( $r=-0.267$ ;  $p=0.031$ ).

The amount of time spent working in the hospital did not correlate with MPO activity, LOOH levels, catalase activity and sulfhydryl groups levels in operating room personnel ( $p>0.05$ ).

## Discussion

Results reveal for the first time in literature that operating room personnel have significantly higher MPO activity and LOOH levels. Furthermore, in the present study, we observed that catalase activity and sulfhydryl groups levels were significantly lower in operating room personnel than in non-operating room personnel. The increased oxidative stress levels and increased MPO activity may be related to the oxidative effect of anaesthetic gases. Therefore, anaesthetic gases may affect MPO activity in operating room personnel.

LOOHs are a by-product of lipid peroxidation. LOOHs are a well-known marker of OS; they form from unsaturated phospholipids, glycolipids and cholesterol by peroxidative reactions during OS.<sup>16</sup> In addition to membrane-bound cholesterol-derived hydroperoxides, oxidized LDL (oxLDL) is the main form of LOOH responsible for the development of OS.<sup>16</sup> OxLDL actively contributes to the progression of

**Table-1:** Demographic characteristics of the two groups in this study.

Parameters	Operating room personnel (n=32)	Non-operating room personnel (n=32)	p
Age (years)	34.6±2.2	35.2±6.1	0.623
Gender (female/ male)	15/17	14/18	0.560
Body mass index (kg/m <sup>2</sup> )	23.3±3.2	24.7±2.5	0.314
Duration of work in the hospital (years)	6.9±3.6	7.1±4.1	0.849
TG (mg/dl)	166.9±114.9	200.4±150.6	0.321
TC (mg/dl)	178.8±49.7	182.3±35.8	0.748
HDL-C (mg/dl)	43.3±10.7	41.1±12.6	0.438
LDL-C (mg/dl)	102.1±42.3	101.2±28.8	0.920

Values are mean ± SD;

TG: Triglyceride

TC: Total cholesterol

HDL-C: High density lipoprotein-cholesterol,

LDL-C: Low density lipoprotein-cholesterol.

**Table-2:** Myeloperoxidase activity, catalase activity, sulfhydryl groups and lipid hydroperoxide levels in operating room personnel and non-operating room personnel.

Parameters	Operating room personnel (n=32)	Non-operating room personnel (n=32)	p
Lipid hydroperoxide (µmol/L)	8.46±1.55	6.28±1.62	0.001
Myeloperoxidase activity (U/L)	63.13±23.97	30.18±9.23	0.001
Catalase (U/L)	70.57±38.29	90.96±19.73	0.009
Free sulfhydryl groups (mmol L-1)	0.43±0.11	0.50±0.07	0.003

Values are mean ± SD.

atherosclerotic lesions and their resulting complications.<sup>17</sup> It is known that a wide variety of drugs either become converted into or stimulate the formation of ROS.<sup>18</sup> OS resulting from an imbalance between radical-generating and radical-scavenging systems is one of the harmful effects associated with anaesthetic gases.<sup>19</sup>

Several studies have suggested that chronic exposure of anaesthetic gas is harmful to operating room personnel despite developed scavenging systems.<sup>3-4,20,21</sup> Sivaci et al.<sup>3</sup> demonstrated that several anaesthetic agents such as desflurane produce ROS and change serum antioxidant levels in operating room personnel. Baysal et al.<sup>4</sup> also investigated OS in operating room personnel, and they also demonstrated significantly increased OS from general anaesthetic agents in operating room personnel. Moreover, Turkan et al.<sup>5</sup> evaluated effect of volatile anaesthetics on OS due to occupational exposure. They found that plasma and erythrocyte antioxidant activity and trace element levels were significantly lower in operating room personnel compared to controls. Izdes et al.<sup>20</sup> investigated deoxyribonucleic acid (DNA) damage, glutathione levels, and total antioxidant capacity in anaesthesia nurses. They showed that occupational exposure to anaesthetic gases induces DNA damage, which may lead to changes in total antioxidant capacity and glutathione levels. More recently, Lee et al.<sup>6</sup> reviewed impact of volatile anaesthetics, such as isoflurane and sevoflurane, on OS and inflammation. On the other hand, Sardas et al.<sup>21</sup> investigated the genoprotective role of antioxidant supplementation in technical anaesthesiology staff working in operating theatres. They demonstrated that occupational exposure to anaesthetic gases induced oxidative DNA damage. They found that DNA damage in operating room personnel was significantly decreased after dietary supplementation with vitamin C and vitamin E for 12 weeks.

MPO, a member of the heme peroxidase superfamily, is contained in azurophilic granules in neutrophils and monocytes. It is released upon leukocyte activation, contributing to innate immunity.<sup>22</sup> MPO is released by leukocytes during inflammatory states and catalyses the formation of several reactive species, including HOCl, and thus plays a role in host defence against microorganisms.<sup>23</sup> The role of MPO, an enzyme secreted from activated neutrophils and monocytes, has attracted scientific interest as a source of free radicals and diffusible oxidants.<sup>24</sup> MPO is linked to OS by its location in leukocytes and its role in catalysing the formation of oxidising agents. MPO activity precipitates atherogenesis. Measurement of MPO in blood may therefore contribute to CVD risk stratification.<sup>25</sup> MPO is secreted upon leukocyte activation and resides in and around endothelial cells.<sup>26</sup> The enzyme and its oxidative

products are present at high levels within human atheromas.<sup>27</sup> Increased levels of MPO and its products have been detected in the plasma as well as in plaques in patients with CVD.<sup>28,29</sup> MPO also mediates the oxidation of LDL-C.<sup>30</sup> and apolipoprotein (apo) AI.<sup>31</sup> Zheng et al.<sup>31</sup> showed that MPO levels correlate with the severity of CAD assessed during angiography.

The source of MPO in plasma is activated leukocytes. The release of MPO and the subsequent formation of ROS may be triggered by several mechanisms.<sup>23</sup> First, inflammation induces recruitment and activation of white blood cells. Second, minimally modified LDL particles in the intima may trigger the influx of monocytes that mature into resident macrophages, some of which express MPO. Third, neutrophils in the blood stream migrate toward and bind to sites of damaged endothelium. The MPO released by these adherent leukocytes is initially bound to the vascular endothelium and is subsequently transcytosed to the subendothelial matrix.<sup>32</sup>

Serum sulfhydryl groups serve as the main antioxidant component of serum and exert their effects via several mechanisms.<sup>33,34</sup> They prevent tissue damage by reacting with free oxygen radicals and lipid peroxides, thereby neutralising them.<sup>33,34</sup> Therefore, the measurement of thiol levels is a good reflection of excess free radical generation.<sup>11</sup> Furthermore, it has been shown that decreased serum sulfhydryl group levels are associated with the severity of CAD.<sup>35</sup> Additionally, catalase constitutes the main antioxidant component of serum and is a member of free radical and ROS scavenging system. It is known that catalase plays an important role in conditions of increased hydrogen peroxide production.<sup>12</sup>

There were several limitations of our study. First, it had a cross-sectional design. However, it was a preliminary study investigating MPO activity and OS in operating room personnel. Second, the sample size was small, and our observations need to be confirmed in a larger study sample. Third, measuring the carotid artery intima-media thickness via ultrasonography is a widely accepted marker of subclinical atherosclerosis. However, in our study, carotid artery intima-media thickness could not be measured. Finally, the relationship between anaesthetic gases and OS could not be examined.

Our findings indicate that operating room personnel exhibit higher OS, which may be due to the oxidative effect of anaesthetics gases. Therefore, anaesthetic gases may affect MPO activity in operating room personnel. Due to the potential effects of anaesthetics gases on OS, it should be useful to minimise exposure to inhalation anaesthetics and to provide better work conditions. Further studies including

larger number of patients are needed to clarify the results.

## Conclusion

Anaesthetic gases may affect MPO activity in operating room personnel. Therefore, it should be useful to minimise exposure to anaesthetics inhalation and to provide better work conditions.

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**Conflict of Interest:** None.

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