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Arylestarase and Oxidative Stress in Operating Room Personnel

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Long-term occupational exposure to trace concentrations of volatile anesthetics is known to have adverse effects on the health of exposed personnel.
Objectives. We investigated paraoxonase-1 (PON1) and arylesterase (ARE), as well as antioxidant status (TAS) and total oxidant status (TOS) levels in anesthesia personnel (AP) who were chronically exposed to inhalation anesthetics, and compared them with levels in a control group.
Material and Methods. We designed a comparative prospective study with 50 female subjects. The first cohort included 25 full-time female workers in operating rooms in two locations in the Antalya Education and Research Hospital in Antalya, Turkey. The control group was comprised of 25 female individuals working in the same hospitals without any work-related exposure to hazardous agents.
Results. Serum ARE activity and TAS levels were significantly reduced ($p = 0.04$ and $p < 0.0001$, respectively), whereas TOS and OSI levels were found to be significantly higher ($p = 0.01$ and $p < 0.0001$, respectively) in AP. However, there were no significant differences in PON1 activity, PON1/HDL-C, ARE/HDL-C, and PON1/ /ARE ($p = 0.30$, $p = 0.5$, $p = 0.1$ and $p = 0.7$, respectively) when the two groups were contrasted.
Conclusions. According to the results of this study, depending on the putative role of PON/ARE in oxidant stress--related diseases, particularly atherosclerosis and cancer, AP might be considered a risk group for the development of atherosclerosis and many other diseases (**Adv Clin Exp Med 2014, 23, 1, 49–55**).

Key words: anesthesia personnel, paraoxonase, arylesterase, oxidative stress, operating room.

Inhalation anesthetics are still important sources of chemical hazards in operating theaters. Many factors influence workplace concentrations of anesthetic gases such as anesthetic procedures, air conditioning, apparatus leakage, fresh gas flow, and functioning of the scavenging system. Long--term occupational exposure to trace levels of volatile anesthetics is known to have adverse effects on the health of exposed staff [1, 2]. The potential detrimental chronic effects of anesthetic gases on neurological, hematological, immunological, reproductive, hepatic and renal systems, plus the possibilities of increased cancer risk, have been subjects of previous research [3, 4]. The pathophysiology of these adverse effects of anesthetic gases is unknown, but several anesthetic agents produce free radicals and change the serum antioxidant levels in patients [5, 6].

Human serum paraoxonase (PON1) and arylesterase/lactonase (ARE) are enzymes that have lipophilic antioxidant features [7]. The first evaluation of the possible physiological role of PON1 came from experiments by Mackness et al. [8]. Since paraoxon and other toxic substances are not present in the human body under normal conditions, other biomolecules would need to be the physiological

substrates of this enzyme. These authors observed, using purified PON1, the prevention against copper-induced oxidation of low-density lipoprotein (LDL), which is provided by HDL. The authors showed that HDL, as well as PON1, prevented lipoperoxide generation during the process of LDL oxidation. This suggested that the enzyme itself might be involved in the protective function attributed to HDL. Studies have provided evidence that PON1 has a broad range of biological substrate specificity such as oxidized cholesteryl esters, oxidized phospholipids, homocysteine-thiolactone, and a number of drugs and pro-drugs [7, 8–10].

Oxidative stress is characterized as an imbalance between the production of free radicals and the elimination of these radicals by protective mechanisms via an agent known as antioxidants. This imbalance inevitably damages significant biomolecules and potentially affects the health of the whole organism [11]. Therefore, studies should focus on the best biomarkers of oxidative stress. TAS, TOS, and OSI biomarkers may be thought to be disease biomarkers and effective supporters in treatment follow-up.

The aim of this study is to research the activity of serum PON1 and ARE in anesthesia personnel (AP) in comparison to healthy controls, and to investigate possible changes concerning oxidative stress.

Material and Methods

Subjects

We designed a comparative prospective study with 50 female subjects. The first cohort included 25 full-time female workers in operating rooms at 2 locations in the Antalya Education and Research Hospital in Antalya, Turkey. The control group was comprised of 25 female individuals working in the same hospitals without any work-related exposure to hazardous agents (e.g. radiation and anesthetic gases). Only the subjects who had spent 5 or more years in the operating rooms were included in this study. For anesthesia, we used isoflurane and sevoflurane, which are used in advised minimum alveolar concentration (MAC) values for patients. Our centers used semi-closed circuit or normal-flow anesthesia.

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. All subjects in both groups filled in a structured questionnaire specifying gender, date of birth, smoking status, 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.

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

Our prevention program does not include routine monitoring of concentrations of in-room volatile anesthetics, but depends on air-conditioning and anesthetic scavenging systems. Hospital operating rooms, equipped with ventilation and anesthetic scavenging facilities, are used for general surgical purposes. The operating theater systems involved ventilation with supplementary fresh air given by a pressure ventilation system (up to 6 air changes/h), pressure and evacuate ventilation systems equipped with ventilation units supplying fresh air to, and discharging contaminated air outside, the operating room (more than 10 air changes/h), and complete air-conditioning systems with laminar air flow (more than 15 air changes/h).

Blood samples were acquired after an overnight fasting state. Serum examples were then separated from the cells by centrifugation; lipid parameters were measured freshly. Remaining serum were stocked at –80°C and used to analyze PON1, ARE, TAS, and TOS levels.

Analytical Methods

Measurement of Paraoxonase and Arylesterase Enzyme Activities in Serum

PON1 and ARE activities were determined by employing commercially available kits (Relassay®, Turkey). The fully automated PON1 activity measurement process is comprised of 2 different reagents. The first reagent is a proper Tris buffer, and it includes calcium ion (Ca+2), which is a cofactor of PON1. Linear rise of the absorbance of p-nitrophenol (PNP), generated from paraoxon, is followed at kinetic measurement mode. Spontaneous hydrolysis of paraoxon was subtracted from the total rate of hydrolysis. The molar absorptivity of PNP is 18.290 M-1 cm-1, and 1 unit of PON activity is equivalent to 1 mol of paraoxon hydrolyzed per L/min at 37°C [12]. Phenyl acetate (PHACET) was used as a substrate to determine ARE activity. PON1, present in the serum, hydrolyses PHACET

to its products, namely phenol and acetic acid. The generated phenol is colorimetrically determined through oxidative linking with 4-aminoantipyrine and potassium ferricyanide. Spontaneous hydrolysis of PHACET was subtracted from the total rate of hydrolysis. The molar absorptivity of the colored complex is 4000 M-1 cm-1, and one unit of ARE activity is equivalent to 1 mmol of PNP hydrolyzed per L/min 37°C [13].

Measurement of the Total Antioxidant Status of Serum

The total antioxidant status of the serum was determined using an automated colorimetric measurement method for total antioxidant status developed by Erel [14]. In this method, antioxidants in the serum (plasma) reduced dark blue-green colored 2, 2′-azino-bis (3-ethylbenzthiazoline-6--sulphonic acid) (ABTS) radical to colorless decreased ABTS form. The alteration of absorbance at 660 nm is related to the total antioxidant concentration of the serum. The method identifies the antioxidative effect of the serum against the potent free-radical reactions initiated by the generated hydroxyl radical. The results are shown as the micromolar trolox equivalent per liter.

Measurement of the Total Oxidant Status of Serum

The total oxidant status of the serum (or plasma) was determined using an automated colorimetric measurement method for total oxidant status developed by Erel [15]. In this method, oxidants present in the serum (or plasma) oxidize the ferrous ion (Fe+2)-chelator complex to ferric ion (Fe+3), which occurs a colored complex with a chromogen in an acidic medium. The color, which can be determined spectrophotometrically, is related to the total amount of oxidant compound available in the serum. The results are shown in point of micromolar hydrogen peroxide equivalent per liter (μmol H2O2 Equiv./L).

Oxidative Stress Index

The percentage ratio of total oxidant status levels to total antioxidant status levels were accepted as oxidative stress index (OSI) [16]. OSI value was calculated according to the following formula: oxidative stress index (arbitrary unit) = total oxidant status (micromolar hydrogen peroxide equivalent per L)/total antioxidant status (micromolar trolox equivalent per L).

Routine Parameters

The levels of serum lipid parameters [total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C)], were measured through using commercially available assay kits (Abbott) with an autoanalyzer (Architect® c16000, Abbott Diagnostics).

Statistical Analysis

Statistical analyses were carried out using MedCalc statistical software (MedCalc, Mariakerke, Belgium). The results were shown as median (95% confidence interval). The importance of the differences between groups was calculated by Wilcoxon test and the Mann-Whitney *U*-test. P values less than 0.05 were accepted as the significance level.

Results

There were no significant differences in age or gender (all female) between AP and controls ($p > 0.05$). Smokers were more common in AP (16%) than in controls (12%), but this did not show a significant difference ($p > 0.05$). Body mass indices (BMI) were similar in both groups ($p = 0.74$). When lipid parameters were compared, total cholesterol (TC) and LDL cholesterol (LDL-C) levels were significantly increased between the AP and the control group [($p = 0.005$) and ($p = 0.006$), respectively], whereas HDL cholesterol (HDL-C) and triglycerides (TG) levels were not statistically significant ($p > 0.05$). Demographic and laboratory findings obtained from AP and controls are summarized in Table 1.

Serum ARE activity and TAS levels were significantly reduced ($p = 0.04$, $p < 0.0001$, respectively), whereas TOS and OSI levels were found to be significantly higher ($p < 0.01$, $p < 0.0001$, respectively) in AP (Fig. 1). However, there were no significant differences in PON1 activity, PON1/HDL-C, ARE/HDL-C, and PON1/ARE ($p = 0.30$, $p = 0.5$, $p = 0.1$, and $p = 0.7$, respectively), when the 2 groups were compared (Table 2). This implies that PON1, ARE, and HDL-C values are independent of each other.

Discussion

Many studies have suggested that chronic exposure to trace levels of anesthetic gas is harmful to operating room personnel despite developed scavenging systems [2, 17–20]. To our knowledge,

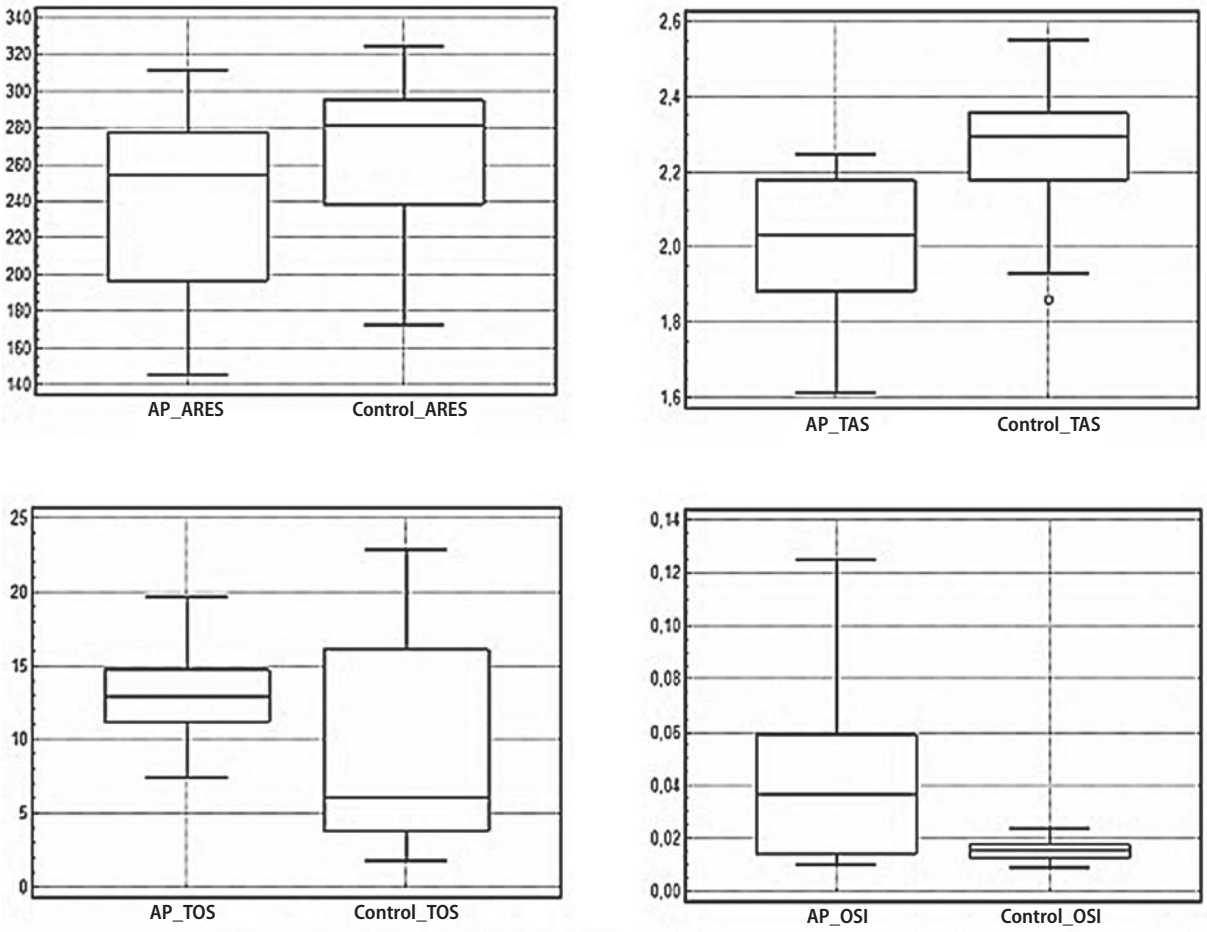

Fig. 1. Serum ARE activity and TAS levels were reduced statistically and significantly, whereas TOS and OSI levels were found to be significantly higher in AP. However, there were no significant differences among PON1 and other parameters

this is the first study to interpret the association between impaired oxidative balance and HDL-related PON1, ARE enzyme activity, and AP. The outcomes of our study showed that the mean TOS and OSI levels were significantly increased; whereas the mean ARE enzyme activity and TAS levels were significantly decreased, in female AP compared to the control female subjects.

Although cancerogenicity, mutagenicity, and many harmful effects are discussed as effects of long-time exposure to anesthetic gases, several review articles have suspected the results of studies, finding positive correlations of the incidence of occupational disease with exposure to the volatile and gaseous substances. Studies have suggested that long-term exposure to trace levels of anesthetic gas is detrimental to operating-room personnel despite developed scavenging systems. Free radicals are one of the harmful effects related to volatile anesthesia [21–23].

Reactive oxygen species (ROS) and free radicals are the results of a cellular metabolism, which is a vital process in the stimulation of signaling pathways in animal cells in response to changes in environmental conditions [24]. Important biomolecules such as proteins, lipids, and DNA are targets for free radicals, and modification of these molecules can raise the risk of mutagenesis [25]. Under sustained environmental stress, ROS are produced over a long time, and thus important damage may occur to the cell structure and functions. In recent years, investigators have raced to confirm that "increased oxidative stress due to the impaired oxidative/antioxidative balance" was the absolute impact in the pathogenesis of human disease, including cancer [26].

PON1 and ARE are enzymes that have lipophilic esterase antioxidant characteristics. PON1 binds to HDL and contributes to the elimination of various chemical compounds such as paraoxon and lipid soluble free radicals from lipid peroxidation. PON1, together with ARE, have been shown to function as a one enzyme [27–29]. Studies have already defined that ARE enzyme activity reflects the antioxidant property of PON1, although it is not directly responsible for it [30, 31].

Table 1. Demographic and laboratory finding of AP and controls were similar except TC and LDL of AP

Parameter	Anesthesia personnel (AP) (n = 25)	Controls (n = 25)	P
Age	40 (37.7–43.6)	34 (28.1–48.2)	NS
Gender	All women	All women	
MWT*	160 h/month	160 h/month	
MWT in OR†	80 h/month	–	
Smoker, (n), (%)	4 (%16)	3 (%12)	NS
BMI (kg/m2)	25.7 (23–27.9)	22.6 (22.9–27.8)	NS
TC (mg/dL)	208 (180.1–230.8)	173 (157.1–185.5)	0,005
TG (mg/dL)	98 (76,1–139,8)	105 (87.7–121)	NS
HDL-C (mg/dL)	49 (41–52.69	47 (39.1–54)	NS
LDL-C (mg/dL)	123 (109.3–158.6)	104.2 (92–118)	0,006

* Mean Working time, † Operating Room, NS – Non significant, TC – Total Cholesterol, TG – Triglycerid, LDL-C – Low Density Lipoprotein Cholesterol, HDL-C – High Density Lipoprotein Cholesterol. Median (%95 CI for median) for all parameters.

Table 2. Oxidative and antioxidative parameters of AP and controls

Parameter	Anesthesia personel (AP) (n = 25)	Controls (n = 25)	P
PON1 (U/L)	120.6 (109.7–146.5)	130.4 (108.5–154)	0.3
ARE (kU/L)	254.5 (207.4–274)	281.3 (240–293)	0.04
TAS (nmol Troloks/L)	2.03 (1.91–2.17)	2.29 (2.19–2.3)	< 0.0001
TOS (μmol H_2O_2 Equiv./L)	12.9 (11.4–14.3)	6.1 (4.01–14.5)	0.01
OSI	6.1 (4.01–14.5)	0.3 (0.1–0.5)	< 0.0001
PON1/HDL-C	2.4 (1.9–3.6)	2.7 (1.9–3.9)	0.5
ARE/HDL-C	5.2 (3.9–6.1)	5.5 (4.8–6.3)	0.1
PON/ARE	0.5 (0.4–0.6)	0.5 (0.4–0.7)	0.7

Median (%95 CI for median) for all parameters.

Interestingly, Tang et al. [32] has shown that low ARE activity is a strong prognostic value for cardiovascular risk. In addition, diminished serum ARE activity provides increased prognostic value, and clinical reclassification of stable subjects are at risk of developing death, myocardial infarction, and stroke.

However, hypercholesterolemia is among the risk factors for atherosclerosis. LDL, the major cholesterol carrier in circulation, can undergo oxidative modification in vascular cells, and cellular uptake of oxidized LDL leads to the generation of ROS [32–34]. LDL oxidation affects its lipid, and oxidative modifications in these molecules are involved in the mechanisms leading to endothelial dysfunction during atherosclerosis [35]. In this study, we found that serum cholesterol and LDL levels of AP patients were significantly increased compared to the control group. These results suggest that AP might be considered a risk group for the development of cardiovascular risk.

Many clinical observations using different methods have reached the conclusion that oxidative stress had increased in AP [2, 17–20]. Serum (or plasma) concentrations of different oxidants and antioxidants can be measured separately in laboratories, but these measurements are labor-intensive, time-consuming, costly, and require specialized personnel. As measuring different oxidants and antioxidant molecules separately is not easy, in order to acquire a simple detection of the oxidative stress in patients, it is enough to only assess the level of TAS and TOS and to calculate the OSI [14, 15].

In conclusion, no biological monitoring of the exposure or direct exposure measurements were performed. In fact, the staff of operating theaters is exposed to many other factors, such as chemical agents other than volatile anesthetics, infected biological material, potential injuries, radiation, artificial lightning, fatigue, and psychosocial stress, which may constitute substantial health hazards. As many of these factors might potentially disturb redox homeostasis, it is risky to assume that the observed changes resulted solely from anesthetic gas exposure, which actually might only be discussed as one possible, though important, reason for the observed phenomena. However, one major limitation of the study is the small number of samples. Apparently, larger studies are needed to constitute the relationship of oxidative stress and other factors in AP.

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