Effect of inhaled anesthetics gases on cytokines and oxidative stress alterations for the staff health status in hospitals

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Abstract

Background

Waste anesthetic gases are few amounts of inhalational anesthesia that escape during its administration. Waste anesthetic gases such as sevoflurane and nitrous oxide can induce toxic effects among health team members working in hospitals. The objective of this study was to identify the harmful effects of upon the waste anesthetic gases exposure on oxidative stress and cytokines of health team members working in hospitals.

Methods

This study involved a control group and waste anesthetic gases exposure group. The exposed group was divided into six subgroups (Surgeons; Surgeon's assistants; Anaesthesiologists; Anesthesiologists' assistants; Nurses and Workers). Serum fluoride, tumor necrosis alpha, interleukin 2 and plasma malondialdehyde, catalase, glutathione peroxidase and superoxide dismutase activities were measured.

Results

A significant elevation was detected in fluoride, tumor necrosis alpha and interleukin 2 levels in anesthesia specialists and anesthesia assistances as compared with the surgeon, surgeon assistants, nurses and workers groups. On the other hand; a significant elevation in levels of malondialdehyde and a significant decline in levels of glutathione peroxidase, catalase and superoxide dismutase in surgeon group as compared with other exposed groups. However, a significant elevation was detected in serum interleukin 4 level among anesthesia specialists as compared with exposed groups.

Conclusions

Exposure to waste anesthetic gases explained a high prevalence of morbidity among operating room personnel which was observed in the present study due to exposure to oxidative stress and cytokines storm. Oxidative stress and inflammatory cytokines are responsible for induction or progression of some diseases. The studied groups (anesthesia specialists and anesthesia assistances) had exposure to high levels of waste anesthetic gases and were more risky to its toxic effects. Accordingly, an education program should be focusing on how to manage these gases to reduce its toxic effects on health staff, and continuous effort evaluating the safety of anesthesia in various aspects is required.

Introduction

During administration, small quantities of vapour and volatile gases (waste anaesthetic gases or WAGs) have been reported to leak into the atmosphere of the operating room from the patient's breathing

apparatus (The Canadian Centre for Occupational Health and Safety (Lawson et al. 2002).

The surgical environment pollution with WAGs is essentially due to three causes: anesthetic techniques, anesthesia workstation, and with or without a scavenging system (Yasny and White 2012). There are many factors play role in occurance of WAGs such as: (1) administration of inhalational anesthesia through face mask; (2) failure to switch off the valve of gas flowmeter and vaporizer; (3) spillage of anesthetic when loading the vaporizer; (4) performing flushing after finishing of surgical procedure to quicken recovery from inhalational anesthesia (common and extremely harmful practice); (5) problems accompanied facial mask such as usage of inappropriate material, wrong size or abnormalities in the patient's airway; (6) leakage of inhalational anesthesia during use of inadequate endotracheal tube cuff or laryngeal mask inflation, or uncuffed endotracheal tube (Lucio et al. 2018).

Consequently, all surgical and support staff (including anaesthetists, nurses, obstetricians and surgeons) are at risk of exposure to these volatiles during anaesthetic administration (Tanko et al. 2014).

Numerous studies, including Vouriot et al. (2005) have demonstrated adverse health impacts following chronic exposure to high levels of inhalational anaesthetics. Moreover, a study by Boucher et al. (1986) revealed a significant prevalence of adverse heart, chest and hepatic problems along with fatigue, headache and irritability.

The imbalance between ROS production and antioxidant defenses is called oxidative stress (Emara et al. 2006; Aldubayan et al. 2019). A range of non-enzymatic and enzymatic antioxidant systems that work to maintain a healthy ROS balance has been described by Lim (2014) and are investigated for their beneficial effects against numerous chronic conditions (Emara et al. 2010). Oxidative stress also plays a significant part in the chronic inflammatory response by directing immune cells to the affected areas (El-Gharieb et al. 2010; Al-Rasheed et al. 2017) and, conversely, high levels of inflammatory mediators due to chronic inflammation promote oxidative stress. While the primary role of inflammation is to defend the individual against harmful stimuli, prolonged inflammation due to stress-induced cellular dysfunction can enhance the risk of chronic illness (Wu et al. 2012).

Greening et al. (2018) reported the beneficial antioxidant and anti-inflammatory properties of isoflurane, which limits the production of chemokines and proinflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) as well as limiting release of prostaglandin E2 (PGE2) by inhibiting cyclooxygenase-2 (COX-2) expression.

According to the ministry of health formulary, drug list in 2014; the most common inhaled anesthetics used in the operating theater of hospitals in Saudi Arabia are Isoflurane and Sevoflurane. One of the most commonly used anesthetics in the Qassim region is Sevoflurane that is widely used in clinical anesthesia because of its safety on the airway and heart. The aim of the present study is to identify the harmful effects of upon the waste anesthetic gases exposure on oxidative stress and cytokines of health team members working in hospitals due to chronic exposure to frequently used inhalation anesthetics.

Subjects And Methods

Study populations

The present study, using a comparative cross-sectional protocol, took place between October 2018 and January 2019 and was performed in several hospitals located in the Qassim region of Saudi Arabia. The study involved a control group of 60 healthy males with no history of exposure to volatile anesthetics (G1) and an exposure group (120 age-matched male operating room workers with histories of potential long-term exposures to WAG). The latter was further subdivided into six sub-groups: Surgeons (G2), Surgeon's assistants [SA] (G3), Anesthesiologists (G4), Anesthesiologist assistants [AA] (G5), Nurses (G6), and Workers (G7).

Occupational histories (working times, work-shifts, work environ, name of anesthetic gases used routinely), along with medical history and smoking status were obtained for all participants in this study. Body mass index BMI calculated as weight (kg) /height (m^2) for all participants. Consent was obtained from each study subject prior to initiation of the study. Criteria used for exclusion from the study included: current smokers, hospital staff with < 1 yr of employment, and any staff with a history of any ongoing disease(s) for which they were currently being treated (and as such would present with a potentially skewed immune status). In addition, the exclusion criteria includes any staff has any immune disease such as atopy and use any medication affecting immune system in the last 6 months.
All participants provided written informed consent and approval for this study was obtained from the college university ethics committee and The Research Ethics Committee at the Ministry of Health for Saudi Arabia.

Collection of blood samples

A blood sample (10 ml) from the hand veins was collected between 8:00 and 9:00 AM after overnight fasting after the work shift. Each blood sample was collected into a silicone-coated tube and then was divided into three parts. The first third was centrifuged (1000 x g, 15 min, 4°C) and the plasma isolated. The remaining materials were either transferred to heparin- or EDTA-coated tubes for eventual isolation of serum. All isolated plasma/serum samples were placed at -20 °C until used for analyses.

Plasma fluoride levels

Plasma levels of fluoride were estimated according to the protocol of Levine et al. (1996) and Şener et al. (2007). In brief, total ionic strength adjustment buffer (TISAB II) and samples were mixed in equal amounts. Magnetic stirrers were used for homogenizing the samples during the estimations. Fluoride was assessed using an ion-selective electrode fitted on an Orion 901 Ion analyzer (Thermo Fisher Scientific, Chelmsford, MA) that also contained fluoride-specific and combination pH electrodes. Standard curves for fluoride were generated in parallel to permit extrapolation of actual plasma levels. The assay was linear over a range of 1-48 µM fluoride, with correlation coefficients equal to 0.999. The limit of quantification was 1 µM fluoride. All samples were assayed in duplicate.

Plasma HFIP levels

Plasma levels of HFIP were estimated according to the protocol of Kharasch et al. (1995) using headspace GC-FID. Total plasma HFIP was estimated by first converting HFIP conjugates to free alcohols by incubating aliquots of each plasma sample with glucuronidase/sulfatase (2,000 U Type HI, Sigma, St. Louis, MO) for 15 hr at 37°C. Each sample was left to cool to room temperature before 200 µL of 3.5 M phosphoric acid was added (injected through septum) to decrease the pH from 5 to 2. Each sample was then vortexed and loaded into the autosampler of an HS40 headspace system (PerkinElmer, Melbourne, FL). The materials were resolved over a capillary column (30 m x 0.53 mm x 3.0 µm film thickness) containing RTX 1701 resin (Restek, Bellefonte, PA). Plasma HFIP levels were ultimately estimated by extrapolation against standard curves (based on areas-under-peak curves) generated with a 2,2,3,3,3-pentafluoro-1-propanol (PFnP) internal standard. All samples were assayed in triplicate.

Enzymatic and non-enzymatic antioxidant assays

The glutathione peroxidase (GSHpx) enzyme activity was detected by the method of Paglia and Valentine (1967) as modified by Lawrence and Burk (1976). The thiobarbituric acid (TBA) method adapted by Satoh (1978) for serum was used to determine the serum lipid peroxides. Super oxide dismutase activity in plasma was determined according to the protocol of Gilles et al. (1976), while the catalase activity was determined according to the protocol of Aebi (1990).

Cytokine assay

The serum concentration of cytokines IL-2, IL-4, and IFN were determined using an ELISA kit supplied by U-CyTech Biosciences (The Netherlands). The coating antibody against each cytokine was diluted and added to each well of a 96-well ELISA plate and incubated overnight at 4°C. The plates were then washed with phosphate buffered saline with tween-20 (PBST) before addition of the blocking buffer and incubation for one hour at 37°C. Stepwise addition of (i) a serum and cytokine stabilizing buffer (CSB) mixture, (ii) biotinylated detector antibody and (iii) horseradish peroxidase (HRP)-conjugated streptavidin polymer was performed, with 1–2 h of incubation at 37°C following each step. The TMB substrate solution was then added and the reaction was stopped after 30 minutes by addition of 2M H_2SO_4. The OD values were obtained using an ELISA reader. Cytokine standards were also prepared and the concentration of each (in pg/ml) obtained via the standard curve.

Statistical analysis

Results are expressed as means ± SD. Data was analyzed using SPSS v.21.0 (SPSS, Inc., Chicago, IL) software. A one-way analysis of variance (ANOVA) was used to discern significant differences among the exposed subgroups (as compared to control). Statistical comparisons between groups were performed using ANOVA, followed by a Dunnett comparison test to compare between the various subgroups. A p-value < 0.05 was considered significant. Pearson correlation coefficients were used to determine potential relationships between blood levels of anesthetic metabolites (fluoride and FHIP) and the oxidative stress

and immune parameters evaluated here. Correlation analysis was carried out with GraphPad Prism version 8.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Results

Control (G1) and WAG-exposed groups (G2-G7) were matched for socio-demographic characteristics (Table 1). There was no significant differences between all of the groups with regard to age, sex (100% of sample was male), marital status, body mass index and/or number of working years.

Plasma fluoride and HFIP levels

Increases in levels of plasma fluoride were detected in all the exposed groups (G2-G7) as compared with the control group (Figure 1a). However, the increase was found to be significant only in the Anesthesiologists and AA (0.44 [± 0.02] and 0.48 [± 0.01], respectively vs. 0.35 [± 0.01]). These values were also significantly greater than those seen in the blood of the Surgeons, SA, Nurses, and Workers.

With regard to plasma HFIP, levels were significantly increased in all the exposed groups (G2-G7) compared with the control group (Figure 1b). In both the Anesthesiologists and AA, the levels were significantly increased as compared with levels in the Surgeons, SA, Nurses, and Workers (Figure 1b). There were no significant differences in plasma HFIP levels between these latter four groups.

Our results showed also that; there was no statistical significant difference between non-exposure (G1) and exposure group (G2-G7) as regards previous diseases.

Changes in plasma oxidative stress markers

Figure (2) showed that; highly statistical significance increase was detected in the levels of plasma MDA in the exposed groups as compared with control group. Highly statistical significance increase was detected in the levels of plasma MDA in the surgeon group as compared with other WAGsexposed groups (Surgeon assistance, anesthesia specialist, anesthesia assistance, nurses and workers). There is no statistical significant difference was detected in the plasma MDA levels between surgeon assistance, anesthesia specialist, anesthesia assistance, and nurses groups (Figure 2).

Figure (2) showed that; highly statistical significance decrease was detected in the levels of plasma GSH, CAT and SOD in the WAGs exposed groupsas compared with control group. Highly statistical significance decrease was detected in the levels of plasma GSH, CAT and SOD in the surgeon group as compared with other exposed groups (Surgeon assistance, anesthesia specialist, anesthesia assistance, nurses and workers) as shown in (Figure 2).No statistical significant difference was detected in the GSH, CAT and SOD CAT levels between surgeon assistance, anesthesia specialist, anesthesia assistance, nurses and workers groups.

Changes in cytokines

Figure (3) showed the changes in tumor necrosis alpha (TNF-α), interleukin 2 (IL2) and interleukin 4 (IL4) levels in different studies groups. A significance increase in the levels of serum TNF-α, IL2and IL4 levels was detected in the WAGs exposed groups as compared with control group. However a significance increase was detected in the levels of serum TNF-$\alpha$and IL2 in both anesthesia specialist and anesthesia assistance group as compared with surgeon, surgeon assistance, nurses and workers groups. On the other hand; a significance increase in the levels of serum IL4levels was detected in the anesthesia specialist group only as compared with other WAGs exposed groups. No statistical significant difference was detected in the levels of serum IL4levels between surgeon, surgeon assistance, anesthesia assistance, nurses and workers groups when compared with control group.

Pearson correlation coefficient of different studied parameters in different studied groups

In Table (2), a significant negative correlation was detected between total fluoride and plasma SOD, catalase, GSH and plasma MDA (Figure 4). On the other hand, plasma MDA had a significant positive correlation with plasma SOD, catalase, and GSH in different studied groups (Figure 4). Table (3) and Figure (5) showed the correlation coefficient (r) of TNF, IL2 and IL4 with Fluoride in different studied groups. Table 2 revealed that Fluoride had a significant negative correlation with TNF and significant positive correlation with IL2. On the other hand, Fluoride had non-significant correlation with IL4 (Table 3& Figure 5).

Discussion

There is a great concern that the operating room personnel might be exposed to health risks due to exposure to anesthetic gases. Health professionals exposed to anesthetic gases are at higher risk of reproductive, neurological, hematological, immunological, hepatic and renal system diseases. However, whether chronic exposure to these gases is hazardous to the health of anesthetic room personnel is still controversial (Dittmar et al.2015).

One of the most commonly used anesthetics in the Qassim region is Sevoflurane that is widely used in clinical anesthesia because of its safety on the airway and heart. This study was conducted in the health team members working in hospitals in the Qassim region, Saudi Arabia. Our study showed that all groups were matched in sociodemographic data with no statistical significant difference between non-exposure (G1) and exposure groups (G2-G7) as regards age, sex (100% males), marital status, the presence of previous diseases and the number of working years. Sevoflurane, isoflurane, and desflurane have 7, 5, and 6 fluoride ions, respectively, and metabolized to produce inorganic fluoride (Sackey et al. 2005). Serum fluoride levels do rise during both short anesthetic and longer ICU duration of use, with sevoflurane displaying higher levels than isoflurane given its greater metabolism and fluoride content (Perbet et al. 2014). In the current study; a significant increase in the levels of plasma fluoride was detected in the exposed groups (G2-G7) as compared with the control group, also in both anesthesia specialists and anesthesia assistance groups as compared with the surgeon, surgeon assistants, nurses and workers

groups. On the other hand; there were no statistically significant difference was detected in the levels of plasma fluoride between the surgeon, surgeon assistants, nurses and workers groups.

Oxidative damage may occur with several drugs and diseases and shows a similar route associated with their development (Chi et al. 2002). Oxidative stress can be generated by an imbalance between the production of oxygen-containing free radicals known as reactive oxygen species (ROS) and their elimination (Elgharabawy et al. 2018). Although ROS is essential for normal metabolism such as killing external harmful factors and maintaining cellular signaling in cells, overproduction of ROS can result in cellular dysfunction (Reuter et al. 2010; Basuony et al 2015).

Various enzymatic and nonenzymatic antioxidant systems contribute to the balance of ROS and have been studied for their protective effect on various chronic diseases (Lim 2014). Anesthetic gases are one group of agents capable of inducing oxidative stress. Various studies have been conducted on oxidative stress and inflammation, mainly focusing on isoflurane and Sevoflurane in rodent models. In human studies, oxidative stress, inflammation, and DNA damage were not affected by isoflurane and sevoflurane in patients undergoing minor incision surgeries. On the other hand, elevated oxidative stress, inflammation, and DNA damage have been observed in patients undergoing major surgeries such as abdominal and orthopedic surgeries, hysterectomy, cholecystectomy, and thoracotomy (Lee et al. 2015). While, Türkan et al. (2011) reported that, sevoflurane caused an increase in the activity of the anti-oxidative enzyme and malondialdehyde in lungs. 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 (Spitz et al. 2004). A possible relationship between occupational exposure to WAGs and oxidative stress is still a relatively unexplored field (Lee et al. 2015). Izdes et al. (2010) reported that the nurses working in operating rooms with no scavenging system, exposed to an average of 14.5 years mainly to isoflurane, sevoflurane, desflurane, and nitrous oxide, had increased breaks in genetic material and reduced enzyme and antioxidant capacity compared to the non-exposed group. Halogenated anesthetics can give rise to reactive metabolites and ROS. In addition, nitrous oxide promotes a reduction in cyanocobalamin, which is followed by the formation of superoxide and hydroxyl radicals/ROS (Costa et al. 2014).

The results of this study indicated that; a significant increase in the levels of lipid peroxidation (plasma MDA) as a marker of ROS concentration and a significant decrease in the levels of plasma GSH, CAT and SOD in operating room staff as compared with the non-exposed group. Also; a highly statistically significant increase in the levels of plasma MDA and a highly statistically significant decrease in the levels of plasma GSH, CAT and SOD were detected in the surgeon group as compared with other WAGs exposed groups (Surgeon assistance, anesthesia specialist, anesthesia assistants, nurses, and workers). Our results did not agree with Malekirad et al. (2005) who reported that a significant increase in lipid peroxidation by thiobarbituric acid-reactive-substances and reduced antioxidant thiol groups in personnel exposed to halothane and N2O for nine years in operation rooms. On the other hand, Türkan et al. (2005) revealed that; Turkish personnel exposed to enflurane, halothane, isoflurane, sevoflurane, and desflurane in operating rooms with partial scavenging system showed reduced plasma GPX and SOD antioxidant

enzymes compared to controls. Also, in agreement with our results, Paes et al. (2014) demonstrated that; medical residents occupationally exposed to waste anesthetic gases induced DNA damage and antioxidant status. Also, our results agree with Lucio et al. (2018) who detected that; genotoxic and oxidative stress effects due to occupational exposure to anesthetic gases among operating room personnel. This demonstrates the requirement for an adequate system for circumventing exposure to a noxious atmosphere. Anesthetic agents may have a direct effect on the anti-oxidant system causing a decrease in the blood flow of the liver, thus leading to a relative increase in the magnitude of free oxygen radical production (Doğru et al. 2017).

These observations suggest that the WAGs exposed increased oxidative stress by lowering the protective mechanism in operating room staff as compared with the non-exposed group. Anesthetic gases and vapors were reported to generate free radicals such as superoxide and hydrogen peroxide which were reported to cause lipid peroxidation.

GSH directly reacts with ROS and neutralize them; many enzymes such as glutathione reductase, and glutathione peroxidase utilize GSH to deactivate ROS. This might be the reason for enhanced lipid peroxidation and depleted GSH content observed in the present study. Türkan et al. (2011) reported that; used the levels of antioxidant enzymes and MDA in the erythrocyte of abdominal surgery patients to demonstrate an antioxidant effect of sevoflurane. In contrast; the safe use of sevoflurane (1.9 %) has also been demonstrated by its lack of influence on the levels of lipid peroxidation and DNA damage (Orosz et al. 2014).

The immune system has many adaptive and dynamic components that are regulated to ensure appropriate, precise and rapid response to a foreign pathogen. A delayed or inadequate immune response can lead to prolonged disease, while an excessive or under-regulated response can lead to autoimmunity. Oxidative stress and inflammation are strongly connected and either one can lead to the other (Costa et al. 2014; Chaoul et al. 2015). Inflammatory cells produce soluble mediators, such as cytokines, which also recruit cells to the site of tissue damage by releasing ROS and further intensifying the inflammatory process (Ferguson 2010). There are no data in the literature concerning the possible alteration of the cytokine profile after occupational exposure to currently used anesthetic gases in operating room personnel. In this study, a significant increase in the levels of serum TNF-α, IL2 and IL4 levels was detected in the operating room staff (exposed groups) as compared with the non-exposed group. Also; a significant increase was detected in the levels of serum TNF-α and IL2 in both anesthesia specialist and anesthesia assistance groups as compared with the surgeon, surgeon assistants, nurses and workers groups. On the other hand; a significant increase in the levels of serum IL4 levels was detected in the anesthesia specialist group only as compared with the surgeon, surgeon assistants, anesthesia assistants, nurses and workers groups.

Similarly, Ke et al. (2008) studied patients receiving anesthesia during abdominal surgery for organ extraction (e.g. the uterus and gallbladder) to find raised cytokine levels in patients receiving isoflurane in comparison to those receiving intravenous anesthesia. In another study by Jin et al. (2013) involving

single-lung ventilation with 6 to 8%, sevoflurane for lung cancer patients undergoing resection indicated exacerbation of numerous pulmonary functions due to the release of inflammatory factors. Many normal functions of the immune system are depressed after exposure to the combination of anesthesia and surgery (Stevenson et al. 1990). Several studies have suggested that impairment of the immune response might increase perioperative morbidity and mortality from infection in susceptible patients (Gunzenhaeuser et al. 1991). In contrast; Takala et al. (2006) and Schilling et al. (2011) who found that the release of TNF-α, IL-8, and IL-1 was significantly decreased during sevoflurane administration compared with total intravenous propofol anesthesia. The anti-inflammatory effects of sevoflurane anesthesia (1 MAC) have also been reported by Schilling et al. (2011), who also, noted a reduction in pulmonary inflammatory cytokines (e.g. IL-6, IL-8, IL-1β, and TNF-α). Orosz et al. (2014) found no effect upon the levels of numerous cytokines, including IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-1β, IFN-γ and TNF-α, during minor surgery under sevoflurane anaesthesia, although a change in the level of IL-6 was observed on the following day due to surgical stress.

We concluded that exposure to WAGs is associated with oxidative stress and changes in cytokines levels among operating room personnel, leading to increased morbidity; so an education programs should be focusing on how to manage these gases to reduce its effects on health staff health status, in addition to continuous effort

Conclusion

Exposure to waste anesthetic gases explained a high prevalence of morbidity among operating room personnel which was observed in the present study due to exposure to oxidative stress and cytokines storm. Oxidative stress and inflammatory cytokines are responsible for induction or progression of some diseases. The studied groups (anesthesia specialists and anesthesia assistances) had exposure to high levels of waste anesthetic gases and were more risky to its toxic effects. Accordingly, an education program should be focusing on how to manage these gases to reduce its toxic effects on health staff, and continuous effort evaluating the safety of anesthesia in various aspects is required.

Declarations

Ethics approval and consent to participate:

All participants provided written informed consent and approval for this study was obtained from the college university ethics committee and The Research Ethics Committee at the Ministry of Health for Saudi Arabia.

Consent for publication:

All authors agreed to publish this article.

Availability of data and materials:

All data and materials are mentioned in the text.

Competing interests:

Non applicable.

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Tables

Table 1. Socio-demographic characteristics and occupational history of the study groups.

Items		G1 (control)	G2	G3	G4	G5	G6	G7
Age (years)		33.5 ± 6.4	34.7 ± 4.8	34.8 ± 7.2	33.5 ± 4.9	35.0 ± 5.0	35.0 ± 3.6	33.9 ± 7.5
Gender	Male	60 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
	Female	0	0	0	0	0	0	0
Marital status	Single	0	0	0	0	0	0	0
	Married	60 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
	Divorcee	0	0	0	0	0	0	0
	Separated	0	0	0	0	0	0	0
Body mass index (kg/m^2)		22.72 ± 0.83	22.70 ± 0.88	22.46 ± 0.88	22.56 ± 0.99	22.58 ± 0.93	22.42 ± 1.07	22.44 ± 0.80
Period of exposure (years)		0	7.5 ± 1.6	6.9 ± 2.0	7.7 ± 1.9	7.1 ± 2.4	7.5 ± 1.4	7.0 ± 2.5

Values expressed as means ± SD. One-way ANOVA/Dunnett test. Value significantly different from control at $^{\#}p < 0.001$.

G1, Control group; G2, Surgeon assistant group; G3, Surgeon group; G4, Anesthesiologist group; G5, Anesthesiologist assistant group; G6, Nurses group; and G7, Workers group.

Table 2: Correlation coefficient (r) of SOD, CAT, GSH and with Fluoride and MDA in different studied groups.

Parameter	SOD	CAT	GSH	MDA
Fluoride	0.08065	-0.03035	-0.09289	-0.1542

****. Correlation is significant at the 0.001 level (2-tailed). ***. Correlation is significant at the 0.005 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 3: Correlation coefficient (r) of TNF, IL2 and IL4 with Fluoride in different studied groups.

Parameter	TNF	IL2	IL4
Fluoride	-0.8135****	0.9242****	-0.2884

****. Correlation is significant at the 0.001 level (2-tailed). ***. Correlation is significant at the 0.005 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Figures

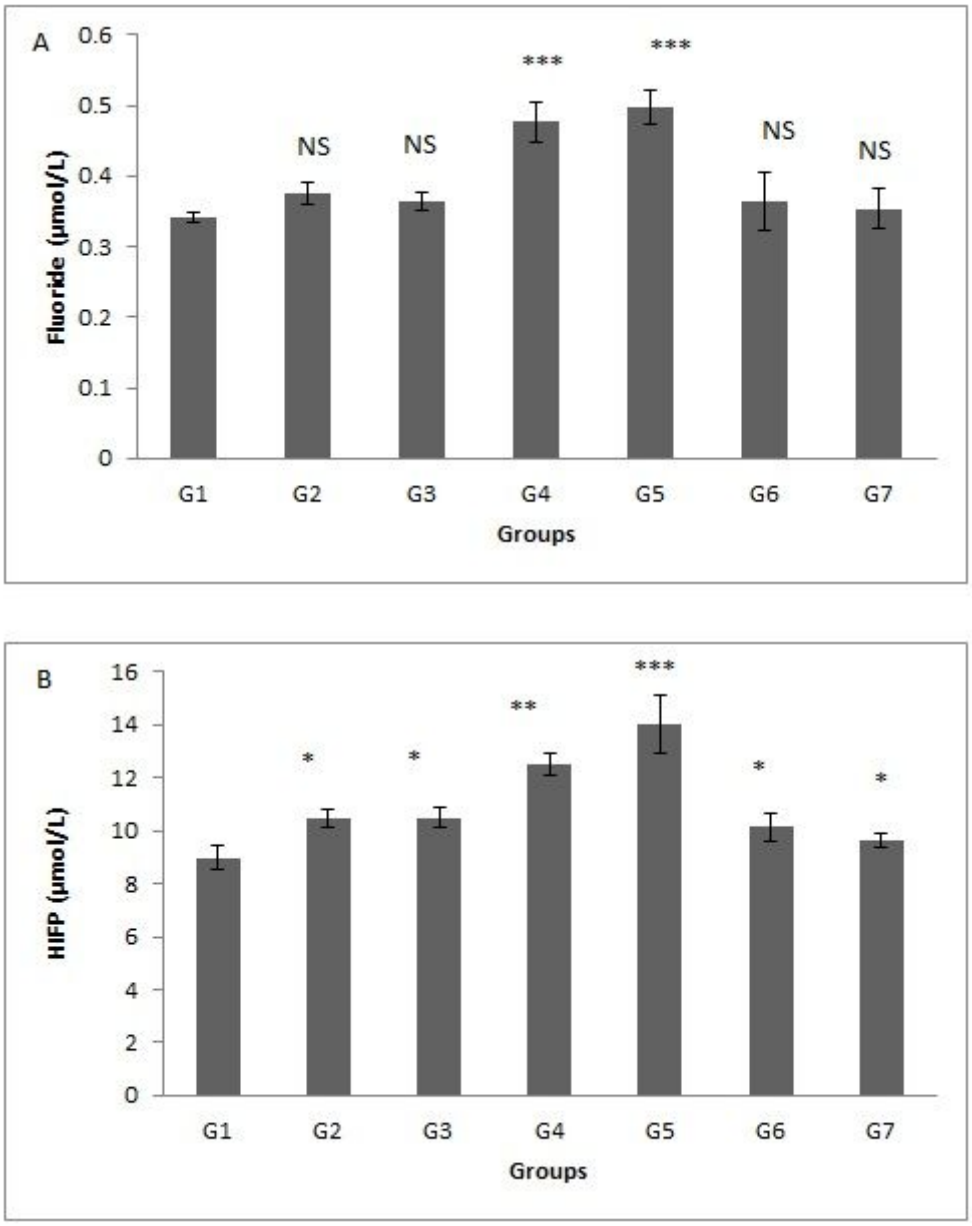


Figure 1

A: Changes in plasma fluoride concentration levels in different groups under study; B: Changes in plasma hexafluoro-isopropanol (HFIP) concentration levels in different groups under study. The significance of difference was analyzed by one – way ANOVA and Dunnett test (compare all vs. control group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at $P < 0.05$. Dunnett test was significant from corresponding control group value at*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

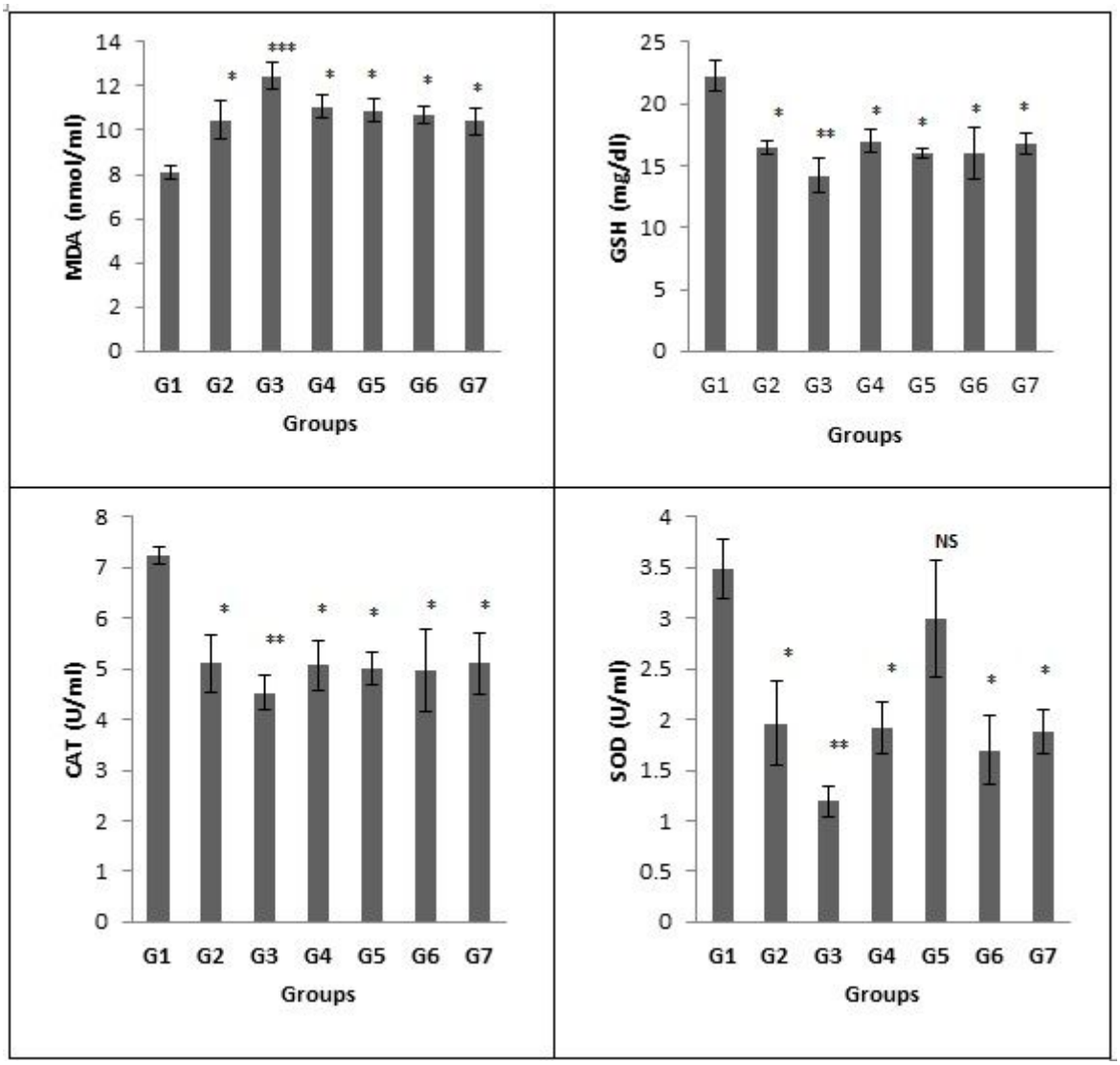
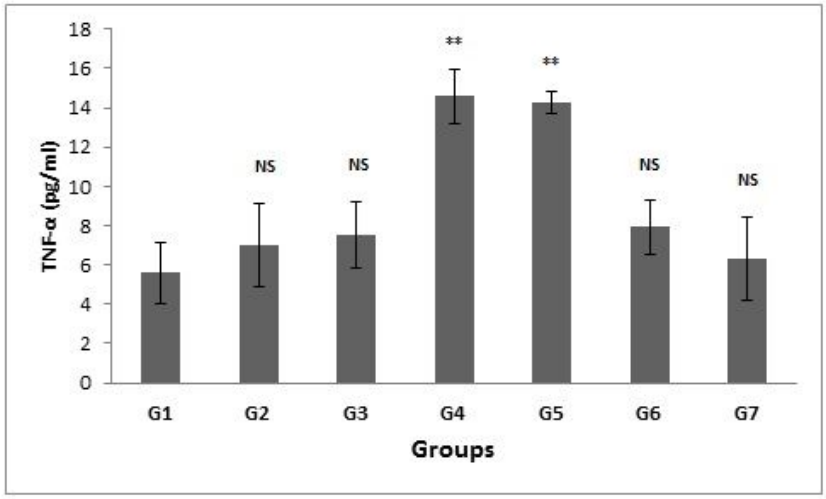
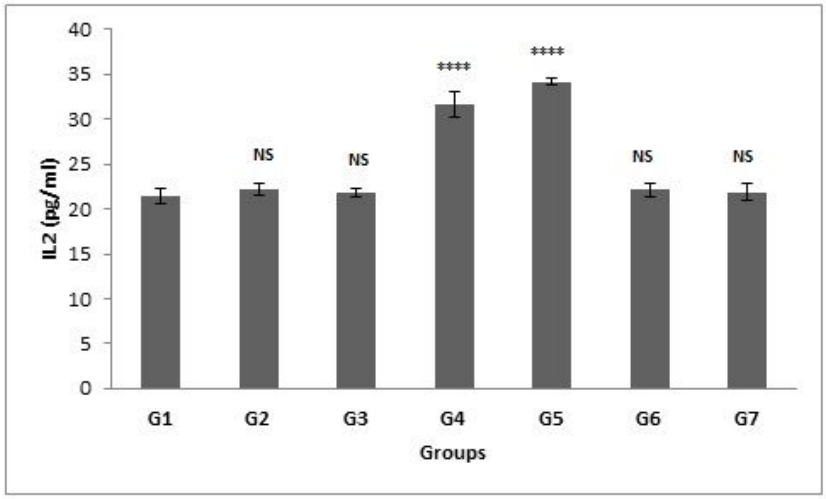


Figure 2

Changes in plasma MDA, catalase, GSH and SOD levels in different groups under study. The significance of difference was analyzed by one – way ANOVA and Dunnett test (compare all vs. control group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at $P < 0.05$.





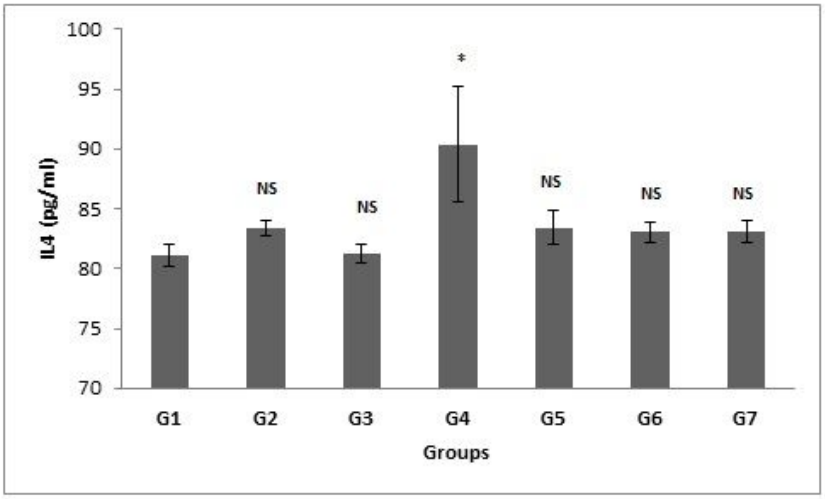


Figure 3

Changes in serum tumor necrosis alpha (TNF-α), interleukin 2 (IL2) and interleukin 4 (IL4) levels in different groups under study. The significance of difference was analyzed by one – way ANOVA and Dunnett test (compare all vs. control group) using computer program. Values are expressed as means ± SEM. one – way ANOVA was significant at $P < 0.05$.

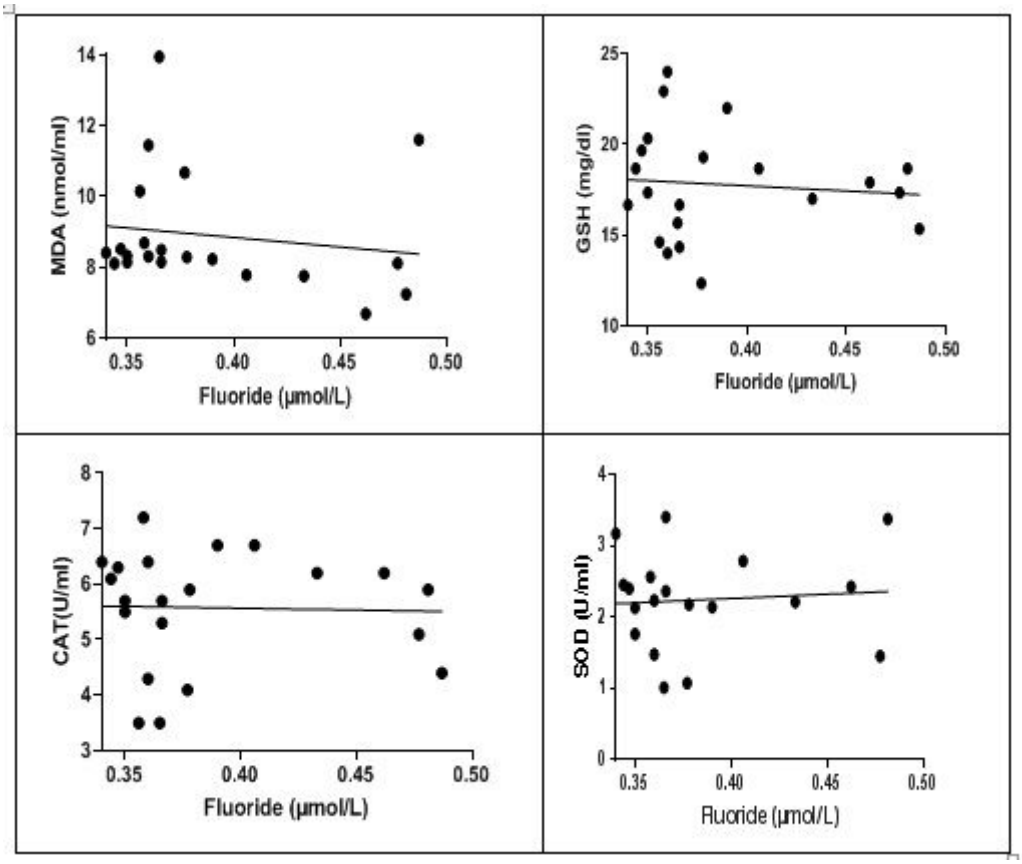


Figure 4

Correlation of plasma MDA, GSH, catalase, SOD with fluoride in different studied groups.

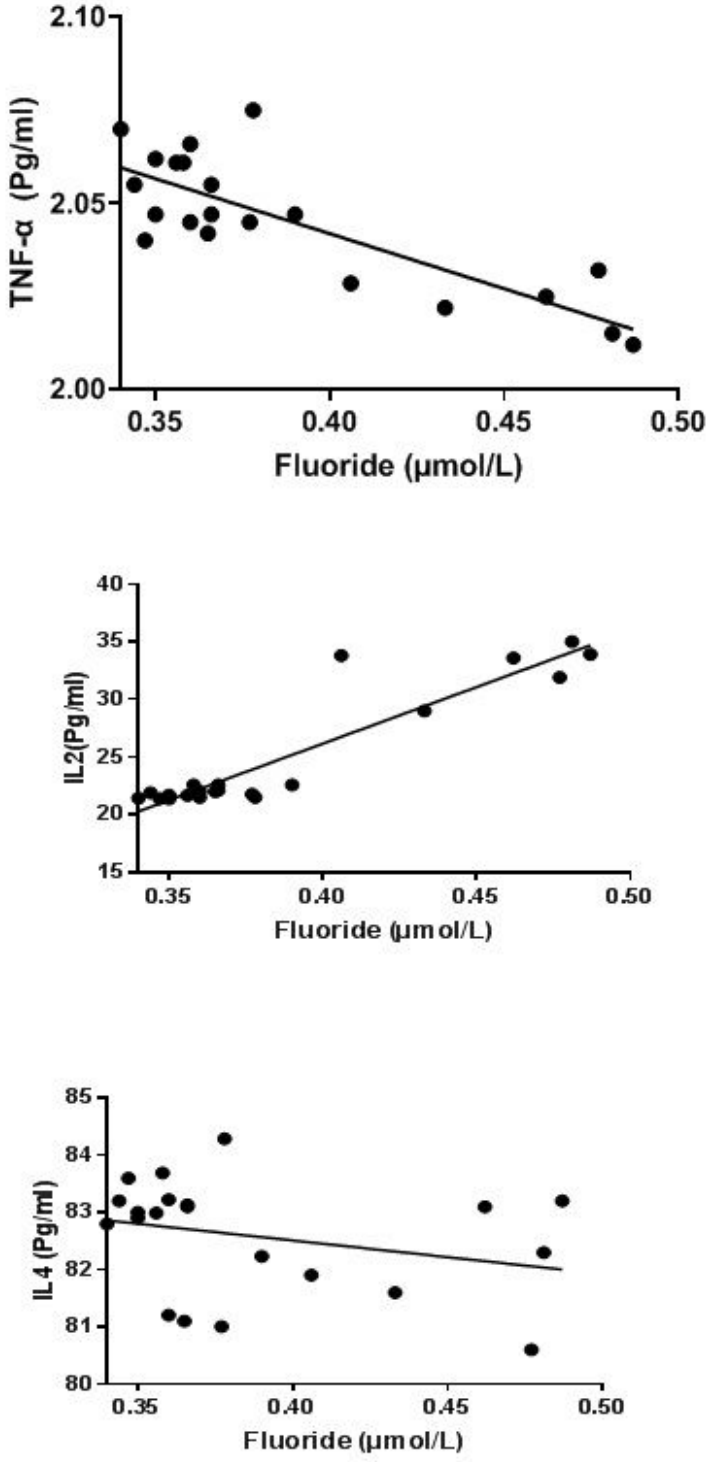


Figure 5

Correlation of serum TNF, IL2, IL4 with fluoride in different studied groups.