Cohort Study

ANNALS OF MEDICINE & SURGERY

OPEN

Immunotoxicity induced by occupational inhalation exposure to waste anesthetic gases: a historical cohort study

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Background: This study was undertaken to ascertain whether long-term occupational exposure to inhalational anesthetic, was associated with any significant alteration in the parameters of immune function.

Materials and methods: This was a historical cohort study in which 30 male participants with at least one year of work experience in the operating room at the time of the study and 30 unexposed referent subjects were investigated. Exposure levels were quantified by measuring the urinary concentrations of nitrous oxide (N2O), isoflurane, and sevoflurane gases by headspace gas chromatography-mass spectrometry. Serum concentrations of interleukin-4 (IL-4), Th2-type cytokines, and interferon-gamma (IFN-γ) were measured by the ELISA method. Additionally, an automated hematology analyzer was used for the white blood cell count and white blood cell differential test. The data were analyzed using SPSS software for Windows version 21.

Results: Mean urinary concentrations of N2O, isoflurane, and sevoflurane *were found to be* 211.57 ± 75.15, 4.06 ± 0.96, and 19.51 ± 12.96 *ppb, respectively.* In simplistic statistical data analysis, significant differences were noted between exposed and control groups as far as the mean serum cytokines levels (IFN-γ, IL-4) were concerned. Furthermore, after adjusting for important confounders, statistical analysis showed that the IFN-γ, IL-4, and the ratio of IFN-γ/IL-4 were significantly higher in the exposed group than in the referent subjects.

Conclusion: These findings provide corroborative evidence to further substantiate the contention that exposure to anesthetics agents (N2O, isoflurane, and sevoflurane) is associated with subtle, subclinical, prepathological changes in the parameters of immune function. The long-term ramification of these changes requires further investigation.

Keywords: immune system, inhalational anesthetics, occupational exposure, operating rooms

Introduction

Currently, inhalational anesthetics (IAs) or anesthetic gases (AGs), particularly, nitrous oxide (N2O), isoflurane, and sevoflurane are used worldwide for the induction and maintenance of anesthesia. AGs especially N2O, beside their benefits in clinical use, are the prominent source of air pollution in the operating rooms (ORs) due to their volatile nature[1–3]. More prevalent use of N2O as compared to other anesthetics, inadequate ventilation systems in the ORs, leakage of gas from N2O cylinders, a lack of regular and periodic inspection to detect leaks in the joints, opening the gas stream before placing the anesthesia mask on the patient's face, and the use of unfitted masks on the patient's face may explain the high atmospheric concentrations of this gas[4].

It is estimated that over 200 000 healthcare workers are potentially exposed to waste anesthetic gases (WAGs) released into the air during medical procedures[5]. The American Conference of Governmental Industrial Hygienists has not set any biological exposure index (BEI) for AGs. However, the

HIGHLIGHTS

- This study was undertaken to ascertain whether occupational inhalation exposure to waste anesthetic gases (WAGs) is associated with any changes in the parameters of immune function.
- Urinary concentrations of nitrous oxide, isoflurane, and sevoflurane were found to be 211.57 ± 75.15, 4.1 ± 0.96, and 16.51 ± 12.96 ppb, respectively.
- The mean values of interferon-gamma and interleukin-4 concentrations were significantly higher in operating rooms personnel with occupational exposure to WAGs than in the nonexposed group.
- Occupational exposure to WAGs is associated with subtle, subclinical, prepathological changes in the parameters of immune function.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Annals of Medicine & Surgery (2023) 85:2313–2318

Received 21 January 2023; Accepted 18 March 2023

Published online 9 May 2023

http://dx.doi.org/10.1097/MS9.0000000000000500

concentrations of unmetabolized AGs or their metabolites in urine have been proposed as BEIs for these compounds. For instance, Accorsi *et al*. have proposed BEI values of 35.5–22.3 ppb N2O in urine to correspond with a threshold limit value of 50 ppm set by American Conference of Governmental Industrial Hygienists and a recommended exposure limit of 25 ppm set by National Institute for Occupational Safety and Health for this gas, respectively. Similarly, a value of 3.6 ppb for urinary sevoflurane has been proposed to correspond with the National Institute for Occupational Safety and Health exposure limit of 2 ppm for this gas[6] . Likewise, Imbriani *et al*. suggested that a urinary N2O level of 25 ppb matches with 50 ppm of the same gas in the air. In a similar fashion, a urinary concentration of 5.3 ppb isoflurane is corresponding with 2 ppm of this gas in the air[7].

Concern has been raised regarding the adverse effects of WAGs following occupational exposure, particularly, their immunotoxicity.

The immune system is a large and complex interconnected network of many different organs that work together to protect the body from harmful agents[8]. The T helper cells (Th cells) are a type of T cell[9]. According to cytokine production, there are different subtypes of Th cells (Th1 and Th2 cells) that play a key role in the adaptive immune system[10]. Th1 cells are characterized by the promoting cell-mediated immune responses. They tend to be pro-inflammatory and are involved in the development of autoimmune diseases. Contrariwise, Th2 cells are associated with humoral-mediated immune. They tend to be anti-inflammatory and are involved in allergic immune responses[11]. IFN-γ and IL-4 are the main Th1 and Th2 cytokines, respectively. IFN-γ, inhibits the expression of IL-4, and vice versa[11]. Variation/imbalance of IFN-γ/IL-4ratio may cause an overactive or a delayed and inadequate immnue response. A delayed-/inadequate immnue response is associated with less protective and defensive effect and prolonged disease status. Whereas, an overactive immune response is associated with the development of autoimmune diseases[12]. Also, imbalances of the ratio of Th cell subsets through their own abnormal activation and decreased activity results in impaired immune regulation, which leads to the occurrence of autoimmune diseases[13].

Numerous cohort, case-control, and experimental studies have established the immunotoxic potential of IAs[14–21]. For example, Matsuoka *et al*.[14] reported a significant positive correlation between the induction of apoptosis in peripheral blood lymphocytes and exposure to IAs (isoflurane and sevoflurane) in dose-dependent and time-dependent manners. Tompa *et al*.[15] demonstrated that chronic exposure to WAGs associated with an increased ratio of lymphocyte subpopulations, lymphocyte activation markers, and leukocyte oxidative burst. Similarly, Koutsogiannaki *et al*.[16] have shown that long-term exposure to isoflurane was associated with impaired neutrophil recruitment and bacterial phagocytosis. Also, N2O, is known to oxide the Co^{+} in vitamin B_{12} to Co^{3+}, resulting in. inhibition of methionine synthetize and vitamin B_{12} deficiency. Vitamin B12 plays an important role in the proper function of immune system. Deficiency of this vitamin can result in changes in immunological parameters[20].

In contrast, some investigators have failed to demonstrate that occupational or nonoccupational exposure to IAs is associated with Immunotoxicity[22–24]. For example, Ziv *et al*. examined a group of 18 anesthesiologists. Hemoglobin levels, white blood cell (WBC) count, B and T lymphocytes and natural killer cells, the number of active T-cells, helper T-cells, suppressor T-cells, and the ratio of helper T-cells to suppressors were determined. Comparing the immunological profiles of anesthesiologists with those of a control group did not reveal any statistically significant differences[23]. Similarly, Aun *et al*.[24] did not find any evidence of change in the percentages of viable or early apoptotic cells in the 26 young physicians after a short exposure to AGs (desflurane, sevoflurane, isoflurane, and N2O).

Although the exact reason(s) for these discrepancies are not known, differences in exposure concentrations, different work histories, occupational and nonoccupational exposure, the issue of cumulative exposure effect, simultaneous exposure to other chemicals, a lack of control of confounders (such as age, sex, smoking, etc.), whether or not the employees wear personal protective equipment, also the type and accuracy of various laboratory tests to assess parameters of immune system function, may explain, at least in part, these discrepancies.

To the best of our knowledge, despite the widespread use of IAs in different Iranian hospitals, to date, no local or national study has been undertaken to evaluate the possible chronic immunotoxic effects of these compounds. This issue along with conflicting results surrounding the possible immunotoxic potential of IAs prompted this study.

Material and methods

Study design and participants

This present historical cohort study was conducted at a large public hospital in Shiraz, the capital city of Fars province in southern Iran, in 2018. The study population was composed of 60 male individuals according to previous studies (divided into two equal groups, exposed and referent)[21,25,26]. The first group consisted of the exposed staff who worked in the ORs (including 5 surgeons, 17 nurses, and 8 technicians) and the second group consisted of nonexposed subjects. The exposed group was further divided into two subgroups depending on the work experience (year): from 1 to 10 years (G1) and more than 10 years (G2).The inclusion criteria for the exposed group were at least one year of exposure to IAs, continuous exposure to IAs over the previous 3 months (except for weekends). The exclusion criteria were the history of past or the presence of current exposure to other chemical contaminants with immunotoxic effects, active immune diseases, use of immunotoxic medications, having blood transfusion up to 3 months before the study and recent exposure to X-ray, and older than 50 years, as age is known to change the immunological parameters[27,28].

The control group consisted of administrative staff and nurses from other wards of the hospital with similar age and sex distribution to the exposed group, without a history of past or current exposure to IAs or chemicals with immunotoxic effects. The inclusion and exclusion criteria for the control group were similar to those of the exposed group except for exposure to IAs.

The study was carried out according to the Helsinki Declaration of 1964, as revised in 2013[29]. Also, this study was conducted in accordance with the strengthening the reporting of cohort, cross-sectional and case-control studies in surgery (STROCSS) criteria for the demonstration of cohort studies[30,31]. The unique identifying number or registration ID for this study is researchregistry8728 (https://www.researchregistry.com/browse-the-registry#home/). All participants filled out and signed an

informed consent form before the commencement of the study. Additionally, they completed a questionnaire that contained questions regarding age, sex, work background, marital status, smoking habits, nature of current and previous works, use of certain medications, health status and history of immune system diseases, height, weight, and other important variables.

Sample collection and assays of immune function

Blood samples were taken from the antecubital vein of the subjects and transferred to two tubes, a disposable tube containing an anticoagulant, (CBC-specific vials) and a disposable tube (without anticoagulant) for the separation of serum. Clot blood samples were centrifuged at 3500 rpm for 5 min to obtain the sera and stored at −80°C until analysis. Serum concentrations of interleukin-4 (IL-4), Th2-type cytokines and interferon-gamma (IFN-γ), Th1-type cytokines were measured by the ELISA method using commercial kits manufactured by Bioassay Technology Laboratory. Additionally, an automated hematology analyzer was used for the WBC count and WBC differential test. An automated Nihon Kohden hematology cell counter, made by Nihon Kohden Corporation in Japan was used for the WBC differential test.

Biological monitoring

Urinary concentrations of IAs including N2O, isoflurane, and sevoflurane were measured by the method introduced by Accrosi *et al*.[32,33].

Statistical analyses

Data were analyzed by the Student's *t*-test, χ^2-test, or Fisher's exact test, ANOVA, and multiple linear regression analyses in SPSS20.

Results

Participant characteristics

Demographic variables, smoking habits, and urine concentration of IAs are presented in Table 1. Only the length of employment of the G2 subgroup ($P = 0.03$) was significantly higher than that of the whole exposed group. No statistically significant differences were noted between exposed and referent subjects as far as demographic variables were concerned. None of the ORs had an active ventilation system or WAGs scavenger.

Urinary concentrations of IAs

The urinary concentrations of N2O were in the range of 55.79–319.91 ppb with a mean of 211.57 ± 75.15 ppb. The geometric mean concentrations of isoflurane and sevoflurane in the urine of the OR staff were 4.1 ± 0.96 (range 0.92–4.72 ppb) and 16.51 ± 12.96 ppb (range 2.13–46.4 ppb), respectively (Table 1). IAs were not detectable in the working atmosphere of the referent subjects.

Indices of the immune system function

Table 2 shows the indices of the immune system function in exposed and unexposed groups. As shown, the serum concentrations of IL-4, IFN-γ, and the ratio of IFN-γ/IL-4 were significantly higher in exposed subjects than in referent individuals.

The association between exposure to IAs and changes in the parameters of immune function was investigated by multiple linear regression analysis. After adjusting for confounders, significant positive correlations were found between the IFN-γ, IL-4, and the ratio of IFN-γ/IL-4 concentrations and exposure to IAs, in that, exposure to IAs resulted in 9.77, 31.46, and 0.49 units of increase in these parameters, respectively (Table 3).

Discussion

This study was undertaken to ascertain whether occupational inhalation exposure to IAs under normal working conditions is associated with any changes in the parameters of immune function.

As shown in Table 1, the urinary concentration of N2O in the exposed individual is several fold (about sixfold) higher than 35.5 ppb, proposed by Accorsi *et al*. and about 8.5 times higher than that of the Italian ORs staff reported by Imbriani *et al*.[6,7].

Likewise, the mean value of sevoflurane was about 4.6 fold higher than the limit set by Accorsi *et al*.[6]. In contrast, the urinary isoflurane concentration was rather lower than the value set by Imbriani *et al*.[7].

Table 1

Comparison of demographic characteristics in the exposed and nonexposed groups (Mean ± SD)

		Exposed group				*P*		
Demographic data		G1 ($n=16$)	G2 ($n=14$)	Total ($n=30$)	Unexposed group ($n=30$)	G1 and Unexposed	G2 and Unexposed	Exposed and Unexposed
Age (year)		35.56 ± 5.85	36.29 ± 7.34	35.92 ± 6.48	35.53 ± 6.01	1**	0.82**	0.82*
BMI (kg/m^2)		26.05 ± 3.11	24.83 ± 2.49	25.48 ± 2.6	24.56 ± 2.72	0.31**	0.98**	0.21*
Work experience (year)		6.81 ± 3.95	15.57 ± 6.28	10.90 ± 6.74	9.83 ± 7.14	0.19**	0.03**	0.55*
Number of smokers (%)	Yes	1 (6)	2 (14)	3 (10)	2 (6)			
	No	15 (94)	12 (86)	27 (90)	28 (94)	0.72†	0.38†	0.50†
Urinary concentrations of anesthetic gases (ppb)	N2O	202.66 ± 72.14	220.47 ± 79.53	211.57 ± 75.15	0.00 ± 0.00	–	–	–
	Isoflurane	3.82 ± 1.17	4.3 ± 0.61	4.1 ± 0.96	0.00 ± 0.00	–	–	–
	Sevoflurane	15.98 ± 9.65	17.82 ± 9.78	16.51 ± 12.96	0.00 ± 0.00	–	–	–

*Independent sample t-test, $P < 0.05$.
**Post hoc test (Dunnott), $P < 0.05$.
†X^2 test, $P < 0.05$.

Table 2

Comparison of immune system parameters in the exposed and nonexposed groups (Mean ± SD)

	Exposed group				*P*		
Indices (units)	G1 ($n=16$)	G2 ($n=14$)	Total ($n=30$)	unexposed groups ($n=30$)	G1 and Unexposed	G2 and Unexposed	total and Unexposed
IL-4 (pg/ml)	24.16 ± 7.03	31.17 ± 5.85	27.43 ± 7.32	17.01 ± 6.99	0.002**	0.0001**	0.0001*
IFN-γ (pg/ml)	55.73 ± 17.69	82.2 ± 20.7	68.08 ± 23.11	36.45 ± 18.47	0.003**	0.0001**	0.0001*
ratio of IFN-γ/IL-4	2.41 ± 0.78	2.63 ± 0.56	2.52 ± 0.68	2.12 ± 0.69	0.34**	0.04**	0.03*
total WBCs (mm^3blood × 10^3)	5.99 ± 1.05	6.00 ± 0.88	5.99 ± 0.95	6.27 ± 1.10	0.81**	0.77**	0.32*
Monocytes%	2.36 ± 0.66	3.15 ± 1.53	2.73 ± 1.20	3.05 ± 2.02	0.26**	0.99**	0.46*
Lymphocytes%	45.95 ± 7.85	47.21 ± 10.10	46.62 ± 8.99	44.22 ± 7.66	0.66**	0.87**	0.27*
Neutrophils%	51.74 ± 11.85	52.32 ± 7.47	52.01 ± 9.88	52.75 ± 7.49	0.98**	0.99**	0.74**

*Independent sample *t*-test, $P<0.05$.
**Post hoc test (Dunnott), $P<0.05$.

A comparison of urinary concentrations of IAs in G1 and G2 subgroups revealed that the employees' exposure to N2O and sevoflurane exceeded their recommended BEIs. Similar findings have been reported by some other investigators[6,34,35].

Our findings indicate that IFN-γ and IL-4 concentrations were significantly higher in ORs personnel with occupational exposure to WAGs than in the nonexposed group. Additionally, the exposed group showed a higher IFN-γ/IL-4 ratio than the control group subjects, indicating that IAs may cause an imbalance in Th1/Th2 cell, towards Th1 dominance.

Similar findings have been reported by some other investigators following repetitive or prolonged exposure to a mixture of WAGs similar to the exposure scenario of our study[12,15,25–27,36,37]. For example, Halawa *et al.*[25] in a study on 22 ORs female nurses and 22 control individuals reported that occupational exposure to low concentrations of WAGs was associated with changes in some parameters of immune system function including percentages of total lymphocytes and lymphocyte subpopulations. Similarly, an investigation of 15 ORs staff with a history of 3-year exposure to mixed IAs (N2O, isoflurane, and sevoflurane) and 15 control participants, found a statistically significant increase in the pro-inflammatory IL-8[26]. Also, Al-Rasheedi *et al.* compared 120 healthy ORs personnel exposed to WAGs with a control group ($n=60$). Serum concentrations of IFN-γ, IL-2, and IL-4 were measured as biomarkers of immunotoxicity. The authors found a relationship between increased levels of serum cytokines and occupational exposure to IAs[12].

Table 3

Association between exposure to anesthetic gases and changes in the immune system parameters using the linear regression model

		SE	CI		
Indices	Beta		lower	UPPER	*P*
IL-4 (pg/ml)	9.77	2.11	5.55	13.96	0.0001*
IFN-γ (pg/ml)	31.46	6.12	19.08	43.84	0.0001*
ratio of IFN-γ/IL-4	0.49	0.2	0.09	0.89	0.02*
total WBCs (mm^3blood × 10^3)	−0.29	0.28	−0.85	0.26	0.29
Monocytes	−0.31	0.45	−1.22	0.59	0.49
Lymphocytes	2.49	2.21	−1.95	6.94	0.26
Neutrophils	−0.93	2.31	−5.51	3.7	0.69

Data for the referent group were used as baseline values.
*Significantly different (linear regression analysis, $P<0.05$).

In contrast, some investigators have failed to demonstrate that occupational or nonoccupational exposure to AGs is associated with changes in the parameters of immune system function. For example, Karakaya *et al.* evaluated the immunological parameters of 32 personnel of the anesthesiology department exposed to low levels of AGs and 20 unexposed referent subjects. No significant differences were noted in the results of immune system parameters such as immunoglobulins (IgG, IgM, and IgA), peripheral blood lymphocytes, and lymphocyte subgroups between both groups[38]. Ji *et al.* compared 28 anesthetists with 28 internal medicine residents. The former had previously been exposed to sevoflurane. They did not observe any significant differences between the apoptosis rates, cell cycles of peripheral blood lymphocytes, and levels of immunoglobulins (IgA, IgM, and IgG) of both groups[39].

It seems that the variation of IFN-γ, IL-4, and imbalance of IFN-γ/IL-4ratio may indicate a change in immune response effect following occupational exposure to IAs among ORs staff.

Historical cohort studies, such as the present study, cannot establish cause-and-effect relationships. Therefore, due to this inherent limitation, it may be argued that the findings of this study may not necessarily be causally linked with exposure to N2O, isoflurane, sevoflurane, or a mixture of them, particularly given the fact that the possible role of chronic stress has not been investigated. While from an epidemiological point of view this is true, it should nonetheless be noted that there are a few lines of circumstantial evidence to indicate that these are very likely to be the direct effects of chronic occupational exposure to IAs. Firstly, none of the exposed participants had any history of preexisting medical conditions. Secondly, exposed subjects did not have any experience of surgery using anesthesia, exposure to X-ray, or any other immunotoxic agent during the course of their employment.

Thirdly, no significant differences were found between the two groups concerning their demographic variables. Fourthly, after adjusting for potential confounders, a statistically significant correlation was found between exposure to IAs and the changes in the parameters of immune function. Fifthly, age is known to change immunological parameter and physiological aging or immunosenescence associated with complex changes and dysregulation of the immune system function through the alterations in cell numbers. The clinical consequences of age-related immune system changes may include an increased risk of infections, malignancy, and autoimmune disorders[40]. The operating staff with an average age and work experience of 35.92 ± 6.48 and

10.90 ± 6.74 years, respectively, are expected to have exposure to AGs for another 20 years or so, making them a more vulnerable group to the immunotoxic potentials of AGs.

Conclusion

These findings provide additional evidence in favor of the notion that occupational exposure to a mixture of IAs by OR personnel is associated with significant changes in the parameters of immune function such as interleukin-4, IFN-γ and the ratio of IFN-γ/IL-4. Long-term ramifications of these subtle, subclinical and prepathological changes require further evaluation.

Ethical approval

The protocol of the study was approved by the university ethics committee (approval number #IR.SUMS.REC.1396.S554) and the study was carried out according to the Helsinki Declaration of 1964 as revised in 2013.

Consent

All participants filled out and signed an informed consent form before the commencement of the study.

Sources of funding

This work was financially supported by the Shiraz University of Medical Sciences, Vice-Chancellor for Research and Technology (grant No.14690), and Iran National Science Foundation (INSF) (Grant number: 96005391).

Conflicts of interest disclosure

The authors declare no conflicts of interest.

Authors contribution

M.N.: conceptualization, investigation, methodology, validation, visualization, supervision, writing – review and editing; F.A.: data curation, investigation, methodology, writing – original draft, preparation, writing – review and editing. M.Z.: writing – original draft, preparation; F.Z.: data curation, formal analysis.

Guarantor

The Guarantor of this study is Fatemeh Amiri.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Acknowledgments

The authors wish to thank all the ORs personnel for their cooperation in this study.

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