Effects of Sevoflurane Exposure on Apoptosis and Cell Cycle of Peripheral Blood Lymphocytes, and Immunologic Function

zhimin ji
Puren Hospital affiliated to Wuhan University
Wanjun Wu
Suzhou Science and Technology Town Hospital
Fan zhou
Puren Hospital affiliated to Wuhan University
Junfang Hu
Puren Hospital affiliated to Wuhan University
Qiuping Xu
Puren Hospital affiliated to Wuhan University
Weibin Yang
Puren Hospital affiliated to Wuhan University
Xueyong Peng
Puren Hospital affiliated to Wuhan University
XInguo Wang
Wuhan Municipal Health Commission
Cheng Zhang
Wuhan Prevention and Treatment Center for Occupational Diseases
Li Li (lili1001@wust.edu.cn)
Puren Hospital affiliated to Wuhan University

Research Article

Keywords: sevoflurane, apoptosis, cell cycle, peripheral blood lymphocytes, immunologic function

Posted Date: January 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-139150/v1

Version of Record: A version of this preprint was published on March 20th, 2021. See the published version at https://doi.org/10.1186/s12871-021-01305-w.

Abstract

Background: Waste anesthetic gases (WAGS) leaked from new-type halogenated inhalational anesthetics such as sevoflurane were reported to pose a risk for the health of operation room personnel. The effects of WAGS on peripheral blood lymphocytes remain yet controversial.

Purpose: The present study was undertaken to examine whether occupational exposure to sevoflurane has detrimental effects on the peripheral blood lymphocytes of exposed medical personnel *in vivo*. Methods: A cohort of 56 medical residents were divided into exposed group (n=28) and control group (non-exposed group) (n=28). Gas chromatograph was used to measure the concentration of sevoflurane in the medical resident's breathing zone during surgeries under inhalation anesthesia in exposure group. The collection time lasted for one hour. Peripheral blood lymphocytes were isolated from venous blood and then apoptosis and cell cycle were analyzed by flow cytometry. EDTA-anticoagulated whole blood was harvested to analyze the lymphocyte subsets by flow cytometry. Immunoglobulins (IgA, IgM, IgG) were quantified by immunoturbidimetry.

Results: The average concentration of sevoflurane in exposed group was 1.03 ppm with a range from 0.03 ppm to 2.24 ppm. Sevoflurane had no significant effect on the apoptosis and cell cycle of peripheral blood lymphocytes in the exposed group relative to the control group (P>0.05). Similarly, there was no significant difference in the lymphocyte subsets and the levels of immunoglobulins (IgA, IgM, IgG) between the two groups (P>0.05).

Conclusion: Occupational exposure to low-level sevoflurane has no significant effect on the peripheral blood lymphocytes of operating room staff, but this conclusion needs to be confirmed by multicenter and long-term follow-up studies with large samples.

Trial registration number and date of registration

ChiCTR2000040772, December 9, 2020 (Retrospective registration)

Background

With the advantage of low solubility in blood and high controllability, inhalation anesthetics are widely used in the operating room (OR), dental clinic, delivery room, MRI room and intensive care unit[1, 2]. Despite many improvements in the anesthesia equipment and the production of safer anesthetic agents during the past decades, inhalation anesthetics inevitably cause waste anesthetic gases (WAGs) in the workplace, including halothane, isoflurane, sevoflurane, desflurane, and nitrous oxide (N_2O)[3, 4]. Furthermore, chronic occupational exposure to the volatile anaesthetics has been reported to have negative influence on the health of hospital personnel since they were introduced into clinical use in the 1990's[1, 5, 6]. Some researchers believe, however, that there are no statistically sound studies identifying the concentrations of anesthetic gases that would exert harmful effects[7–9]. Moreover, the effects of the inhalational anesthetics on lymphocyte apoptosis remain controversial. Matsuoka *et al* found that

sevoflurane induced apoptosis in peripheral lymphocytes in dose-dependent and time-dependent manners *in vitro*[10]. Loop *et al* also documented that sevoflurane induced T cells apoptosis *in vitro*[11]. On the contrary, Aun et al failed to reveal significant differences in the percentages of viable and early apoptotic cells detected by flow cytometry between medical residents with and without brief occupational exposure[12]. Thus, the present cohort study was undertaken to ascertain whether occupational exposure to sevoflurane has harmful effects on the peripheral blood lymphocyte of medical personnel exposed to inhalational anesthetics *in vivo*.

Methods

Study design and occupational exposure

This study was approved by the Human Medical Research Ethics Review Board, Puren Hospital (prll2018001) prior to its initiation. All subjects who participated in this study provided written informed consent, and we adhered to the stringent ethical requirements for research on human subjects. All participants answered a standardized and detailed questionnaire that included demographic data, medical history, lifestyle and anesthetic exposure before each blood sample was collected. Pregnant women, individuals with any chronic infectious or inflammatory disease, and participants using illicit substances, medications, vitamins, and/or antioxidant supplements, and those who had recently been exposed to radiation (within six month), and those who had medical or family history of blood diseases were excluded from the study to avoid the effects of possible confounding factors. The exposed group consisted of 28 anesthetists mainly exposed to sevoflurane at least 24 months of exposure. The control group comprised 28 residents from internal medicine who were not exposed to waste anesthetic gases or other pollutants. The exposed group was age- and sex-matched with the unexposed group. The biological sampling was performed from 2019 to 2020.

Measurement of waste anesthetic gases in the breathing zone of medical residents during surgeries

There were 13 operation rooms which have an average area of 25 square meters without a scavenging system but with vertical laminar flow in Puren Hospital. All the medical residents from the exposed group worked in the 13 operating rooms in the hospital. All the WAGs were collected in the breathing zone of medical residents during surgeries under inhalation anesthesia in all the operating rooms. The collection time lasted for one hour. The measurements were performed by using a GilAir-5 sampler (Sensidyne, USA) and Agilent 7890BGas Chromatograph (Agilent Technologies, USA) according to the instruction of the manufacturer. The averages gas concentration of sevoflurane was calculated and shown in ppm. Measurement requirement was in accordance with National Institute Occupational Safety and Health (NIOSH) approved procedure[13].

Peripheral blood lymphocyte preparation

EDTA anticoagulated venous blood samples were collected from all participants. Peripheral blood lymphocytes were separated within subsequent 10-20 min with a standard method by centrifugation over lymphocyte separation medium at 400×g for 20 min (lymphoprep^{TM}, STEMCELL technologies, Canada). The cells were washed with PBS (250×g, 10 min). Subsequently, 5×10^5 /ml. PBMCs suspended in the PBS were used in all the experiments.

Assessments of apoptosis

The percentages of viable (annexin-/propidium iodide-PI-) and early apoptotic (annexin+/PI−) cells were quantified by using annexin V-fluorescein isothiocyanate (FITC) staining, which was used to detect phosphatidylserine that is externalized in the early phases of apoptosis. Annexin V is an important marker of early apoptosis in which changes in externalized phosphatidylserine levels occur prior to DNA fragmentation[14] .We used the Annexin V-FITC Apoptosis Analysis Kit according to the manufacturer's instructions (Tianjin Sungene Biotech Co., Ltd., China); 1×10^5 mononuclear cells were labeled, incubated in the dark for 15 min and immediately sorted by flowcytometry (BD FACSAria^{TM}ш, USA). Marked annexin V-FITC staining (green) was analyzed using the FL-1channel, while PI staining (red) was analyzed using the FL-2 channel. The data were analyzed by using the flowjo software on a BD FACS Aria™ cytometer (BD BioSciences, USA).

Cell cycle analysis

Cell cycle was analyzed by flow cytometry according to a previous report[15]. Briefly, cells (1× 10^6) were harvested and washed with 10 ml PBS by centrifugation for 5 min at 300×g, and then re-suspended in 0.5 ml PBS. They were fixed by adding 4.5 ml pre-chilled 70% ethanol while vortexing. After incubation for 2 hours at 4°C, residual ethanol was eliminated by centrifugation for 5 min at 300×g. Supernatants were removed and discarded. Cells were washed with 5 ml FACS buffer twice by centrifugation for 5 min at 200×g. Cells were stained using 0.5 ml propidium iodide staining solution and kept in dark. Afterwards, they were incubated for 20 min at room temperature and the fluorescence analyzed using the ModFit LT software on BD FACSCalibur cytometry (BD, BioSciences USA).

Analysis of subpopulations of lymphocyte by Flow cytometry

Freshly collected EDTA-anticoagulated whole blood were processed within 2 h of collection by ten-color flow cytometry (BD FACSCanto^{TM}, USA) to analyze the lymphocyte subsets as previously described[16, 17]. The lymphocyte subsets were identified using the following monoclonal antibodies: anti-CD3-APC-

H7, anti-CD8-PerCP-cy5.5, anti-CD4-BV605, anti-CD25-PE, anti-CD56-BV510, anti-CD19-APC, and anti-□□-BV421 from BD Biosciences; anti-CD28-PE-CY7 and anti-CD127-FITC from Biolegend. The cell suspension was incubated at room temperature in the dark for 30 min. Red blood cells were removed using 500 µl of lysis buffer at room temperature in the dark for 10 min. Finally, the cells were analyzed by using FACS Canto flow cytometry and flowjo software (BD BioSciences, USA).

Immunoglobulin quantification by immunoturbidimetry

The blood of the elbow vein was collected into coagulation-promoting blood vessel, and the content of immunoglobulins (IgA, IgG, IgM) in the serum was detected by Immunoturbidimetric Assay according to the instructions of immunoglobulin assay kit (Shanghai Fosun Long March Medical Science co., LTD) on an automatic biochemical analyzer (beckmancoulterAU5800, USA)

Statistical analysis

Data were analyzed using an SPSS software version 25. The sample size for this study was calculated based on a pilot study, with a test power of 80% and level of 5% of significance (mean expected differences of 7.02 and 7.85 standard deviations between two groups), and was determined to be 52 subjects. By adding a 10% sample shedding rate, finally, the sample size was 56. Independent student's t-test was used for comparing the mean of quantitative variables and chi-square for comparing the mean of qualitative variables. *P*-Values less than 0.05 were considered significant.

Results

Demographic characteristics of the study populations

The demographic characteristics of the exposed and the control groups are shown in Table 1. No statistically significant differences were noted between the two groups as far as these variables (age and sex) were concerned ($p > 0.05$).

Table 1
The characteristics of the study populations

	Control group (n = 28)	Exposed group (n = 28)	*P* value
Age(years)			0.183*
Mean ± SD	34.04 ± 6.03	32.14 ± 4.34	
Range	25–48	27–46	
Sex (male/female)			
male	17	15	0.589#
Female	11	13	
Duration of exposure(years)			
Range		2–20	
* Independent sample t test. #Chi-square test.			

Concentration of sevoflurane in exposed group is lower than the standard of United States National Institute of Occupational Health and Safety (NIOSH)

After analyzing the concentration of sevoflurane collected from the breathing zone of all residents in the exposed group, we found that the average concentration of sevoflurane was 1.03 ppm with a range from 0.03 ppm to 2.24 ppm. The value was lower than the limit recommended by NIOSH.

Sevoflurane exposure has no effect on apoptosis and cell cycle of Peripheral blood lymphocyte

As shown in Table 2 and Figs. 1 and 2, we found there was no significant differences in the apoptosis and cell cycle of the peripheral blood lymphocytes between control group and exposed group ($p > 0.05$). In addition, sevoflurane exposure had no significant effects on cells at G1 phase, G2 phase and S phase in the exposed group compared with the control group ($P > 0.05$). Only the proportion of S phase and apoptosis rate in the exposed group was slightly higher than that in the control group ($P > 0.05$).

Table 2
Comparison of apoptosis rate and cell cycle between control group and exposed group

Cell cycle	group	N	mean	SD	t	*P* value
G1% of total cell number	control	28	84.79	11.36	1.11	0.27
	exposed	28	81.49	11.0		
Mean DNA content of G1 phase	control	28	48.54	2.46	1.62	0.11
	exposed	28	47.50	2.33		
G2% of total cell number	control	28	7.02	3.17	0.06	0.95
	exposed	28	6.98	2.56		
Mean DNA content of G2 phase	control	28	97.41	5.98	1.83	0.07
	exposed	28	94.60	5.47		
S% of total cell number	control	28	6.89	9.92	-0.79	0.44
	exposed	28	9.07	10.83		
apoptosis rate	control	28	6.26	4.87	-0.542	0.59
	exposed	28	7.03	5.73		

Sevoflurane exposure has no impacts on subpopulations of lymphocytes

There was no statistic difference in all subpopulations of lymphocytes between the exposed group and the control group ($p > 0.05$, Table 3, Figs. 3, 4 and 5). Specifically, the percentages of helper T cells, killer T cells, Th to Tc ratio, immature CD4 + T cells, regulatory T cells, mature NK cells and γδT cells in the exposed group were comparable to those in the control group. The results showed that the percentages of mature CD4 + T cells, mature CD8 + T cells, NK cells, immature/mature CD4 + T cells and B cells in the control group were slightly lower than those in the exposed group, with no significant differences found. The percentages of T cells, immature CD8 + T cells, immature/mature CD8 + T cells, immature NK cells, immature/mature NK cells and TNK cells were slightly higher in the control group than in the exposed group ($P > 0.05$).

Table 3
Comparison subpopulation of Lymphocyte between control group and exposed group

subpopulation of lymphocyte	group	N	mean	SD	t	*P* value
T cells %of lymphocyte	control	28	64.18	8.76	1.81	0.08
	exposed	28	59.91	8.86		
Helper T cells %of T cells	control	28	40.83	8.75	0.72	0.47
	exposed	28	39.17	8.43		
Killer T cells %of T cells	control	28	49.29	8.93	-0.67	0.50
	exposed	28	50.92	9.18		
Th to Tc ratio	control	28	0.89	0.37	0.68	0.50
	exposed	28	0.82	0.33		
ImmatureCD4 + T cells %of CD4 + T cells	control	28	92.53	4.06	0.62	0.54
	exposed	28	91.70	5.96		
Mature CD4 + T cells %of CD4 + T cells	control	28	7.47	4.06	-0.62	0.54
	exposed	28	8.32	5.96		
Immature/mature CD4 + T cells	control	28	17.41	11.08	-0.67	0.51
	exposed	28	20.03	17.43		
Immature CD8 + T cells %of CD8 + T cells	control	28	63.64	9.49	1.16	0.25
	exposed	28	60.69	9.64		
Mature CD8 + T cells %of CD8 + T cells	control	28	36.35	9.49	-1.16	0.25
	exposed	28	39.31	9.64		
Immature/mature CD8 + T cells	control	28	1.92	0.70	1.22	0.23
	exposed	28	1.70	0.67		
Regulatory T cells %of CD4 T cells	control	28	2.96	0.58	0.21	0.83
	exposed	28	2.93	0.63		
B cells %of lymphocyte	control	28	12.68	3.42	-1.59	0.12
	exposed	28	14.36	4.41		
NK cells %of lymphocyte	control	28	16.19	7.25	-1.52	0.14
	exposed	28	18.86	5.87		
Immature NK cells %of NK cells↓	control	28	10.18	7.08	0.60	0.55

subpopulation of lymphocyte	group	N	mean	SD	t	*P* value
	exposed	28	9.14	5.98		
Mature NK cells %of NK cells↓	control	28	89.81	7.08	-0.53	0.60
	exposed	28	90.74	5.92		
Immature/mature NK cells	control	28	0.12	0.10	0.80	0.43
	exposed	28	0.10	0.08		
TNK cells %of lymphocyte	control	28	11.32	4.63	0.86	0.39
	exposed	28	10.15	5.44		
γδT cells %of T cells	control	28	7.85	4.33	0.16	0.88
	exposed	28	7.67	4.31		

Sevoflurane exposure has no influence on the levels of immunoglobulins

Immunoglobulins, including IgA, IgM, and IgG, were not significantly different in the exposed group from those in the control group ($p > 0.05$ Table 4 and Fig. 6).

Table 4
Comparison of Immunoglobulin quantification between control group and exposure group

Immunoglobulin quantification(g/l)	group	N	mean	SD	t	P value
IgG	control	28	11.20	1.85	0.80	0.43
	exposed	28	10.85	1.37		
IgM	control	28	1.94	0.88	1.41	0.17
	exposed	28	1.64	0.75		
IgA	control	28	2.53	0.80	-0.53	0.60
	exposed	28	2.65	0.92		

Discussion

Our study demonstrated that the concentration of sevoflurane in the breathing zone of medical residents working in the operating room was lower than the standard of NIOSH; actually chronic sevoflurane exposure had no significant effect on the apoptosis and cell cycle of peripheral blood lymphocytes of exposed medical staff; differences in the subpopulations of lymphocytes and the levels of

immunoglobulins (IgA, IgM, IgG) were not significant between the exposed medical residents and unexposed ones. These findings indicated that average concentration of the WAG sevoflurane exposed in the actual operating room environment does not exceed the standard concentration limit. Furthermore, low and chronic occupational exposure to sevoflurane has no significant effect on apoptosis and cell cycle of the peripheral blood lymphocytes of operating room staff. Naturally, lymphocyte subgroup analysis and immunoglobulin quantification (IgA, IgM, IgG) did not change significantly in exposed group. Consistently, a recent study by Aun did not found significant differences in the percentages of viable and early apoptotic cells detected by flow cytometry among medical residency with brief occupational exposure *in vivo.* The study was performed before the medical residency program (no exposure; the physicians served as their own controls) and again after 1/2 year and 1 year of exposure. [12]. Though previous studies found sevoflurane induced apoptosis in peripheral lymphocytes *in vitro*[10, 11], we believe that the findings from experiments *in vitro* do not be directly applied to actual conditions *in vivo*. The reason for this inconsistency may be speculated as follows: (1) such low concentration of sevoflurane is not enough to cause remarkable influences on peripheral blood lymphocytes. (2) Some regulatory mechanism *in vivo* may correct the effects of sevoflurane exposure on peripheral blood lymphocyte. (3) Regeneration of peripheral blood lymphocytes alleviates the degree of injury from sevoflurane. Early in 1978, Ferstandig demonstrated only high levels of anesthetics and long-time exposure cause significant histotoxicity on cells and animals[7]. Therefore, it is meaningless to use high concentration of inhalation anesthetics predicting the effects of WAGs. Consequently, in unscavenged operating rooms, low concentration of sevoflurane exposure less than standards should be nontoxic[7]. Additionally, there are no statistically sound studies proving that trace concentrations of anesthetic gases exert harmful effects[7]. Meanwhile, Byhahn believe, under modern air conditioning, personnel's occupational exposure is low, and inhalational anesthesia is safe from the standpoint of modern workplace laws and health care regulations[18]. Our study further supports the conclusions mentioned above by analyzing the actual effects of sevoflurane on apoptosis and cell cycle of peripheral blood lymphocytes, and immunologic function *in vivo*, and revealed that exposure to low level of sevoflurane has no significant harmful effects on peripheral blood lymphocyte of operating room staff.

Of course, there are some limitations in this study. First of all, all the subjects were limited to one hospital, and the environment in one hospital operating room could not be directly generalized to other hospitals. Secondly, lack of long-term observation of the dynamics of the subjects. Multicenter and long-term follow-up studies with large samples are warranted to further confirm our results. Research about the effect of exposure to WAGs on cognitive function in addition to immune function is also worth investigation in the future.

Conclusion

In the modern laminar flow operating room, medical personnel's occupational exposure to sevoflurane does not exceed standard limits. Chronic occupational exposure to sevoflurane (less than limit of exposure) was found to have no significant harmful effect on the peripheral lymphocytes of operating room staff.

Declarations

Funding:

This study was financially supported by Hubei Province Key Laboratory of Occupational Hazard Identification and Control, Wuhan University of Science and Technology, 2018 Annual Open Fund (OHIC2018K02).

Competing interests:

The authors declare that they have no competing interests.

Ethics approval and consent to participate:

This study was approved by the Human Medical Research Ethics Review Board, Puren Hospital (prll2018001) prior to its initiation. All of the procedures were performed in accordance with the Declaration of Helsinki and relevant policies in China. All subjects who participated in this study provided written informed consent.

Consent for publication:

Not applicable.

Availability of data and material:

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Code availability:

flowjo software, ModFit LT software, SPSS software

Authors' contributions:

Zhimin Ji and Li Li designed and took charge of the whole study including perform all experiments and manuscript writing. Xinguo Wang and Cheng Zhang were responsible for measuring the concentration of sevoflurane. Wanjun Wu collected and organize data, and make statistical analysis, and manuscript modification. Qiuping Xu and Xueyong Peng were responsible for subject recruitment and information

registration. Fan Zhou and Weibin Yang were responsible for apoptosis and cycle experiments. Wanjun Wu and Junfang Hu was responsible for analysis of lymphocyte subsets and immunoglobulin assay.

Acknowledgments:

I would like to thank my colleagues from the Department of Anesthesiology and Internal Medicine of Puren Hospital for their support to this study.

References

1. Souza KM, Braz LG, Nogueira FR, Souza MB, Bincoleto LF, Aun AG *et al*, et al.Occupational exposure to anesthetics leads to genomic instability, cytotoxicity and proliferative changes. Mutation research. 2016;791-792:
2. Deng HB, Li FX, Cai YH, Xu SY, et al.Waste anesthetic gas exposure and strategies for solution. Journal of anesthesia. 2018;32:2.
3. Vodicka P, Musak L, Fiorito G, Vymetalkova V, Vodickova L, Naccarati A, et al.DNA and chromosomal damage in medical workers exposed to anaesthetic gases assessed by the lymphocyte cytokinesis-block micronucleus (CBMN) assay. A critical review. Mutation research. 2016;770:Pt A.
4. Lucio LMC, Braz MG, do Nascimento Junior P, Braz JRC, Braz LG, et al.[Occupational hazards, DNA damage, and oxidative stress on exposure to waste anesthetic gases]. Revista brasileira de anestesiologia. 2018;68:1.
5. Kunze N, Weigel C, Vautz W, Schwerdtfeger K, Junger M, Quintel M *et al*, et al.Multi-capillary column-ion mobility spectrometry (MCC-IMS) as a new method for the quantification of occupational exposure to sevoflurane in anaesthesia workplaces: an observational feasibility study. Journal of occupational medicine and toxicology. 2015;10:
6. Braz MG, Souza KM, Lucio LMC, Di Renzo GCC, Feliciano LM, Marcondes JPC *et al*, et al.Detrimental effects detected in exfoliated buccal cells from anesthesiology medical residents occupationally exposed to inhalation anesthetics: An observational study. Mutation research Genetic toxicology and environmental mutagenesis. 2018;832-833:
7. Ferstandig LL, et al.Trace concentrations of anesthetic gases: a critical review of their disease potential. Anesthesia and analgesia. 1978;57:3.
8. Szyfter K, Stachecki I, Kostrzewska-Poczekaj M, Szaumkessel M, Szyfter-Harris J, Sobczynski P, et al.Exposure to volatile anaesthetics is not followed by a massive induction of single-strand DNA breaks in operation theatre personnel. Journal of applied genetics. 2016;57:3.
9. Amiri F, Neghab M, Shouroki FK, Yousefinejad S, Hassanzadeh J, et al.Early, Subclinical Hematological Changes Associated with Occupational Exposure to High Levels of Nitrous Oxide. Toxics. 2018;6:4.

10. Matsuoka H, Kurosawa S, Horinouchi T, Kato M, Hashimoto Y, et al.Inhalation anesthetics induce apoptosis in normal peripheral lymphocytes in vitro. Anesthesiology. 2001;95:6.
11. Loop T, Dovi-Akue D, Frick M, Roesslein M, Egger L, Humar M *et al*, et al.Volatile anesthetics induce caspase-dependent, mitochondria-mediated apoptosis in human T lymphocytes in vitro. Anesthesiology. 2005;102:6.
12. Aun AG, Golim MA, Nogueira FR, Souza KM, Arruda NM, Braz JRC *et al*, et al.Monitoring early cell damage in physicians who are occupationally exposed to inhalational anesthetics. Mutation research. 2018;812:
13. National Institute of Occupational Safety and Health (NIOSH):NIOSH Pocket Guide to Chemical Hazards. Washington, DC, U.S.; 1994.
14. van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP, et al.Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry. 1998;31:1.
15. Kim KH, Sederstrom JM, et al.Assaying Cell Cycle Status Using Flow Cytometry. Current protocols in molecular biology. 2015;111:
16. Kotfis K, Biernawska J, Zegan-Baranska M, Zukowski M, et al.Peripheral Blood Lymphocyte Subsets (CD4+, CD8+ T Cells, NK Cells) in Patients with Cardiovascular and Neurological Complications after Carotid Endarterectomy. International journal of molecular sciences. 2015;16:5.
17. Lubbers J, van Beers-Tas MH, Vosslamber S, Turk SA, de Ridder S, Mantel E *et al*, et al.Changes in peripheral blood lymphocyte subsets during arthritis development in arthralgia patients. Arthritis research & therapy. 2016;18:1.
18. Byhahn C, Heller K, Lischke V, Westphal K, et al.Surgeon's occupational exposure to nitrous oxide and sevoflurane during pediatric surgery. World journal of surgery. 2001;25:9.

Figures

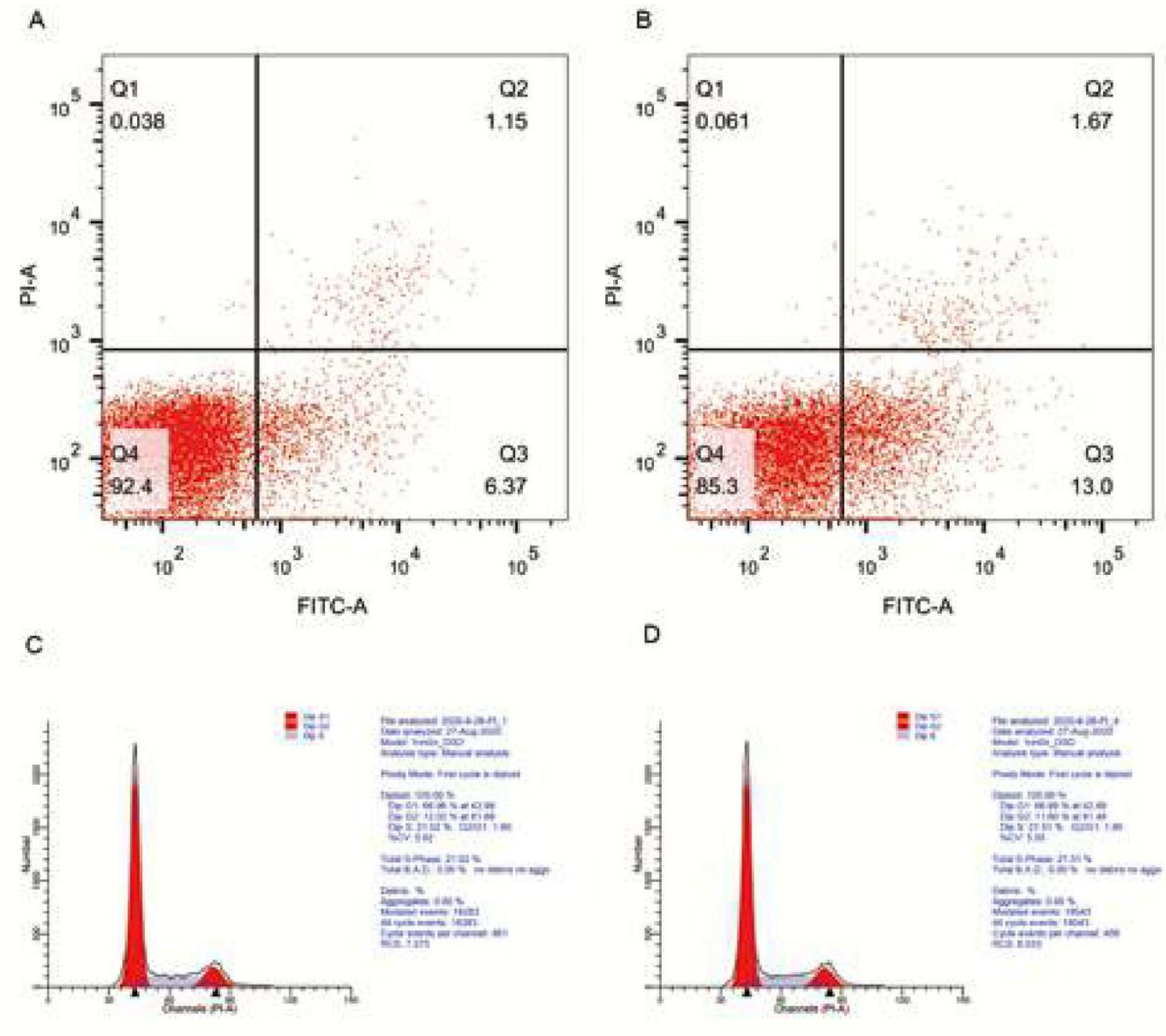

Figure 1

Apoptosis and Cell cycle of peripheral blood lymphocyte analyzed by flow cytometry. A and C are representative pictures of control group. B and D are representative pictures of exposed group.

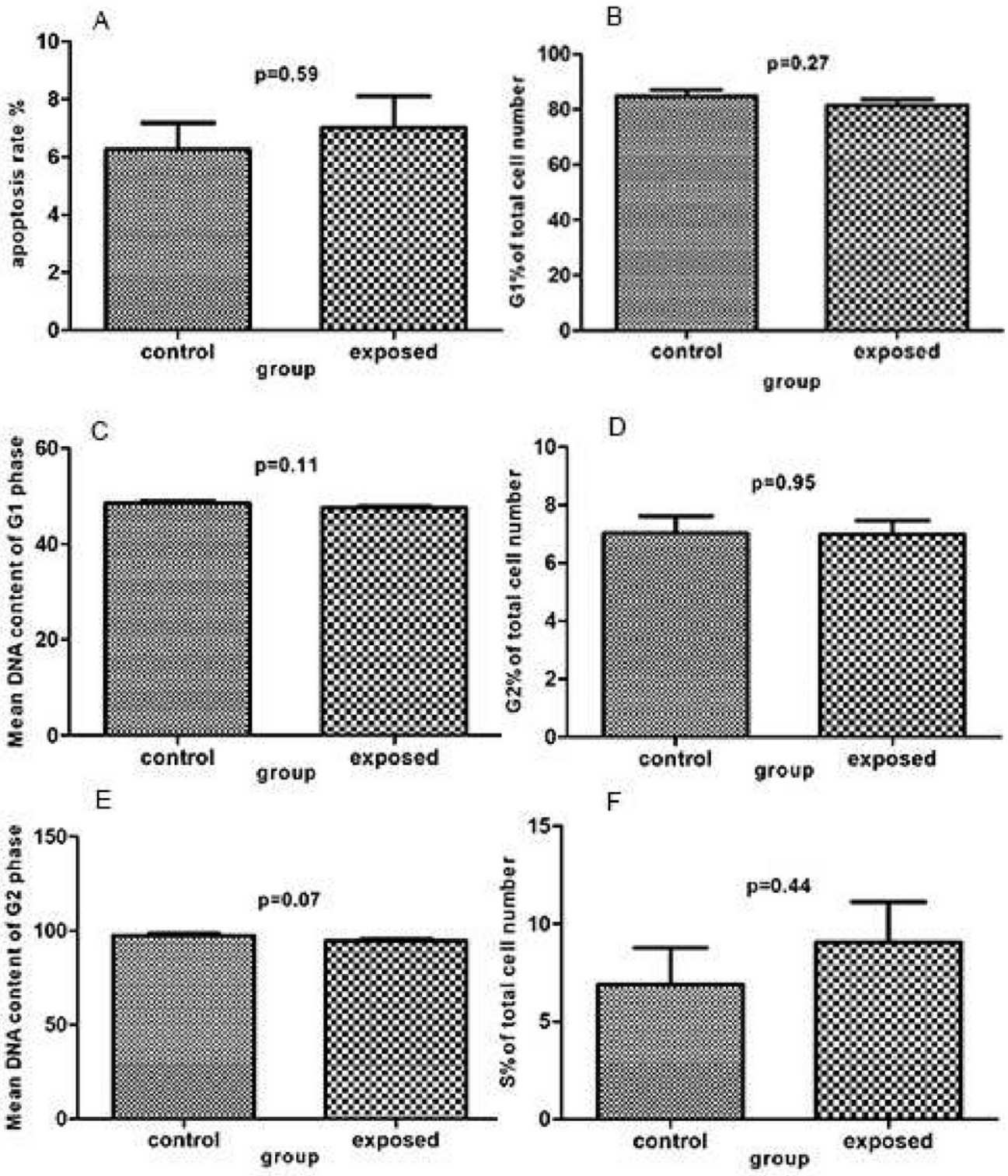

Figure 2

cartogram of apoptosis rate and cell cycle. A is comparation of apoptosis rate of control group and exposed group. B, D and F represent percentage of G1 phase, G2 phase and S phase of cell cycle. C and E display mean DNA content of G1 phase and G2 phase respectively.

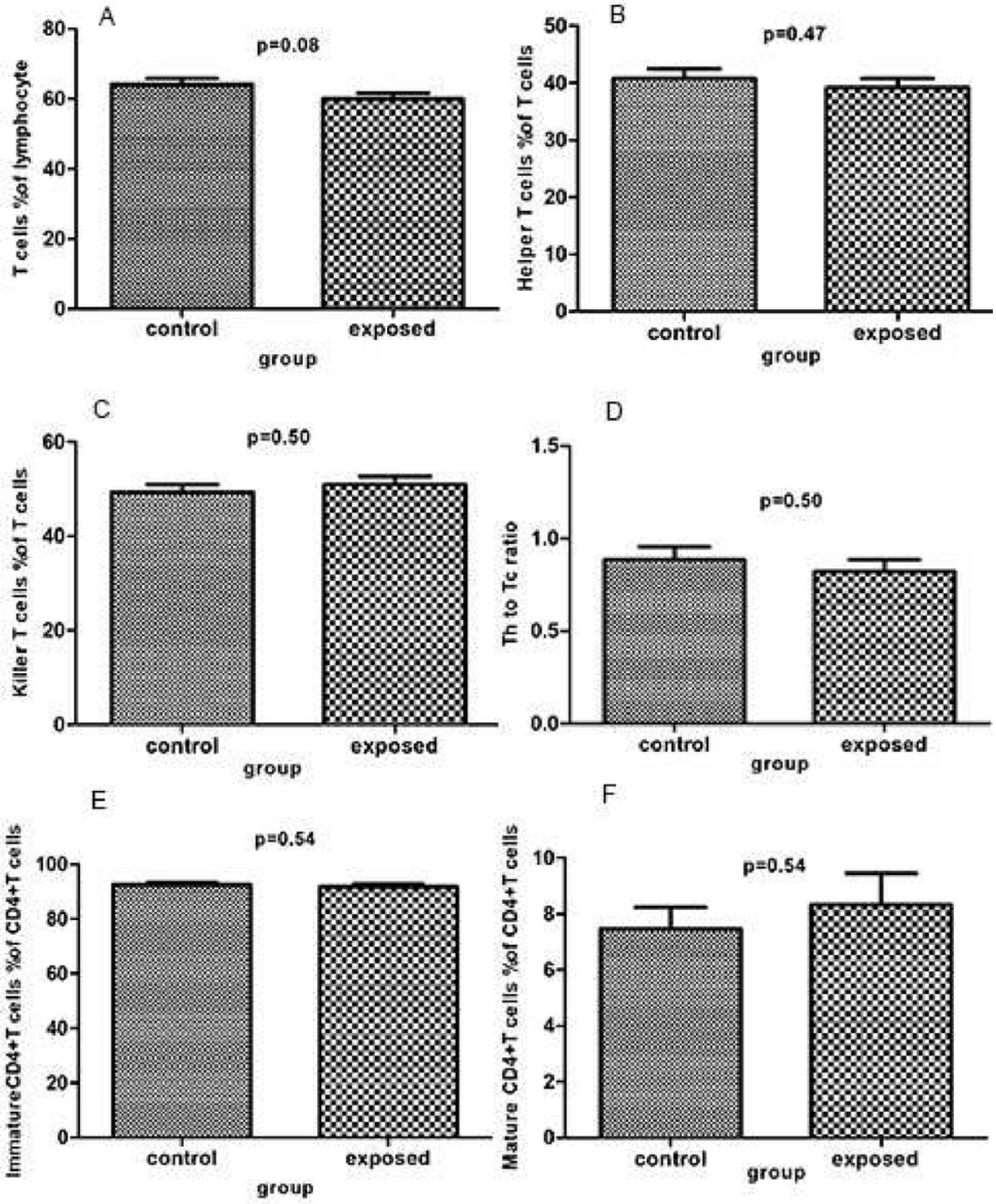

Figure 3

Analysis of subpopulation of lymphocyte by flow cytometry. A-F reveal various subpopulation of lymphocyte as follows: T cells %of lymphocyte, Helper T cells %of lymphocyte, Killer T cells %of T cells, Th to Tc ratio, ImmatureCD4+T cells %of CD4+T cells, Mature CD4+T cells %of CD4+T cells.

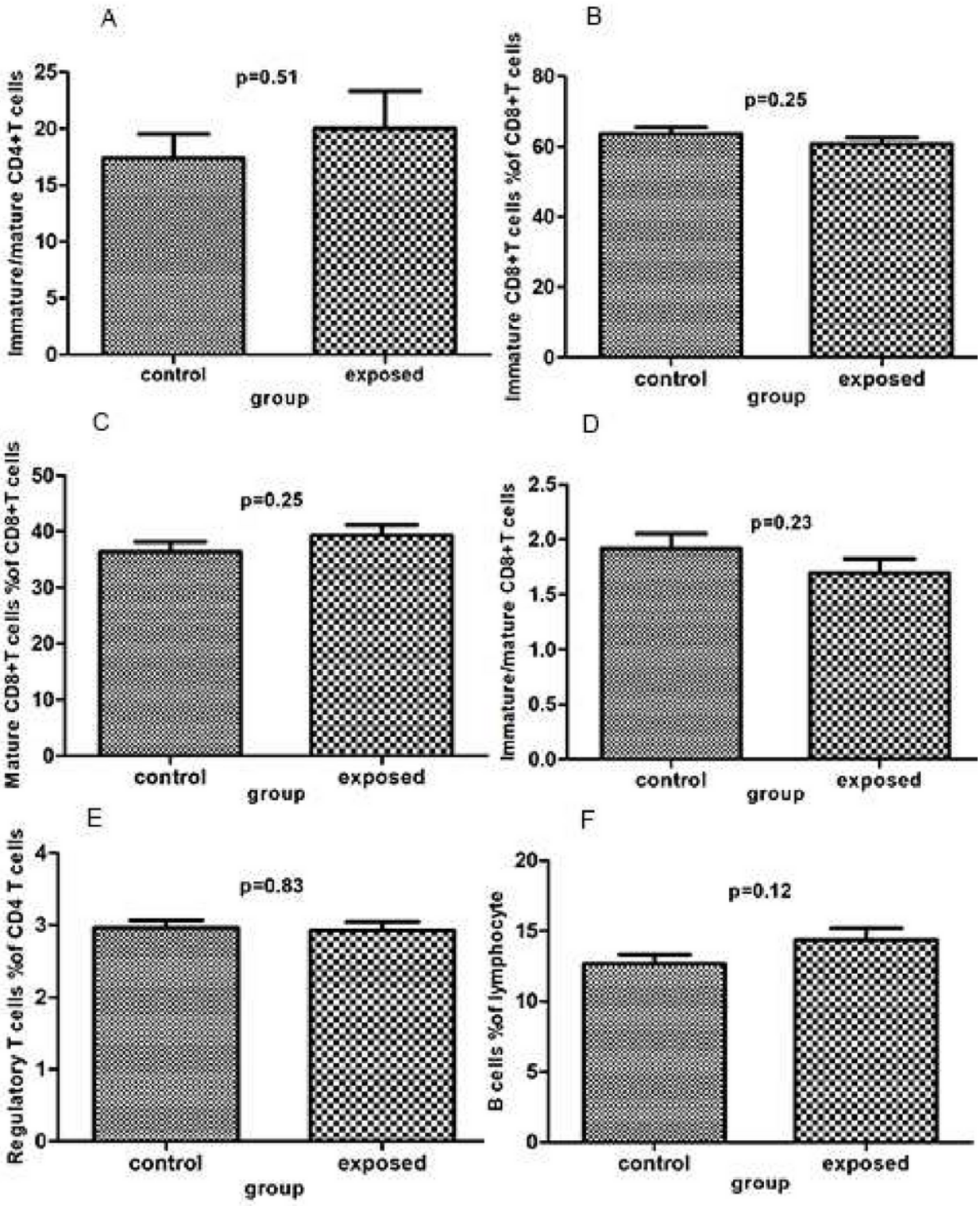

Figure 4

Analysis of subpopulation of lymphocyte by Flow cytometry. A-F show various subpopulation of lymphocyte as follows: Immature/mature CD4+T cells, Immature CD8+T cells %of CD8+T cells, Mature CD8+T cells %of CD8+T cells, Immature/mature CD8+T cells, Regulatory T cells %of CD4 T cells, B cells %of lymphocyte.

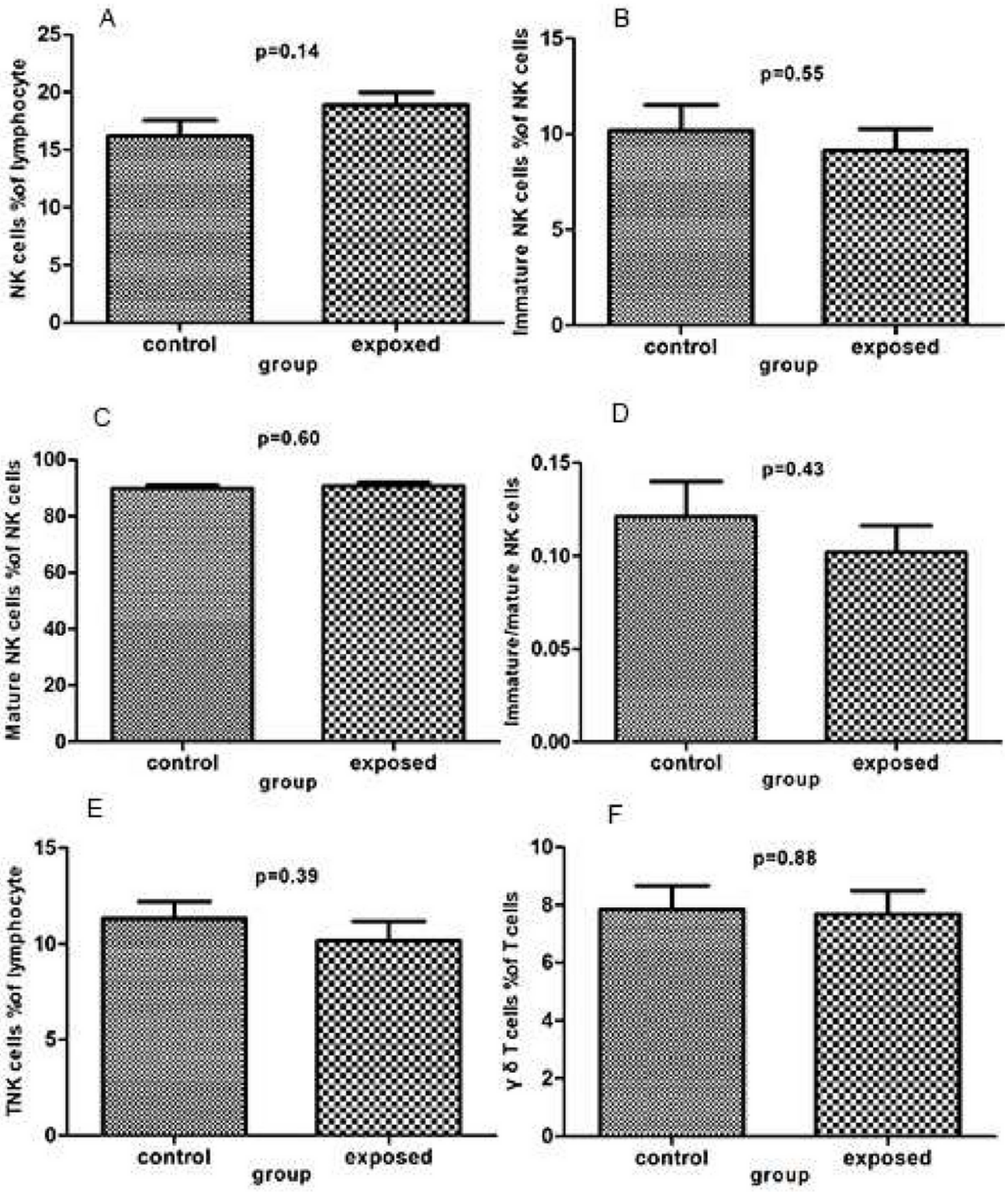

Figure 5

Analysis of subpopulation of lymphocyte by Flow cytometry. A-F show various subpopulation of lymphocyte as follows: NK cells %of lymphocyte, Immature NK cells %of NK cells, Mature NK cells %of NK cells, Immature/mature NK cells, TNK cells %of lymphocyte, γδT cells %of T cells.

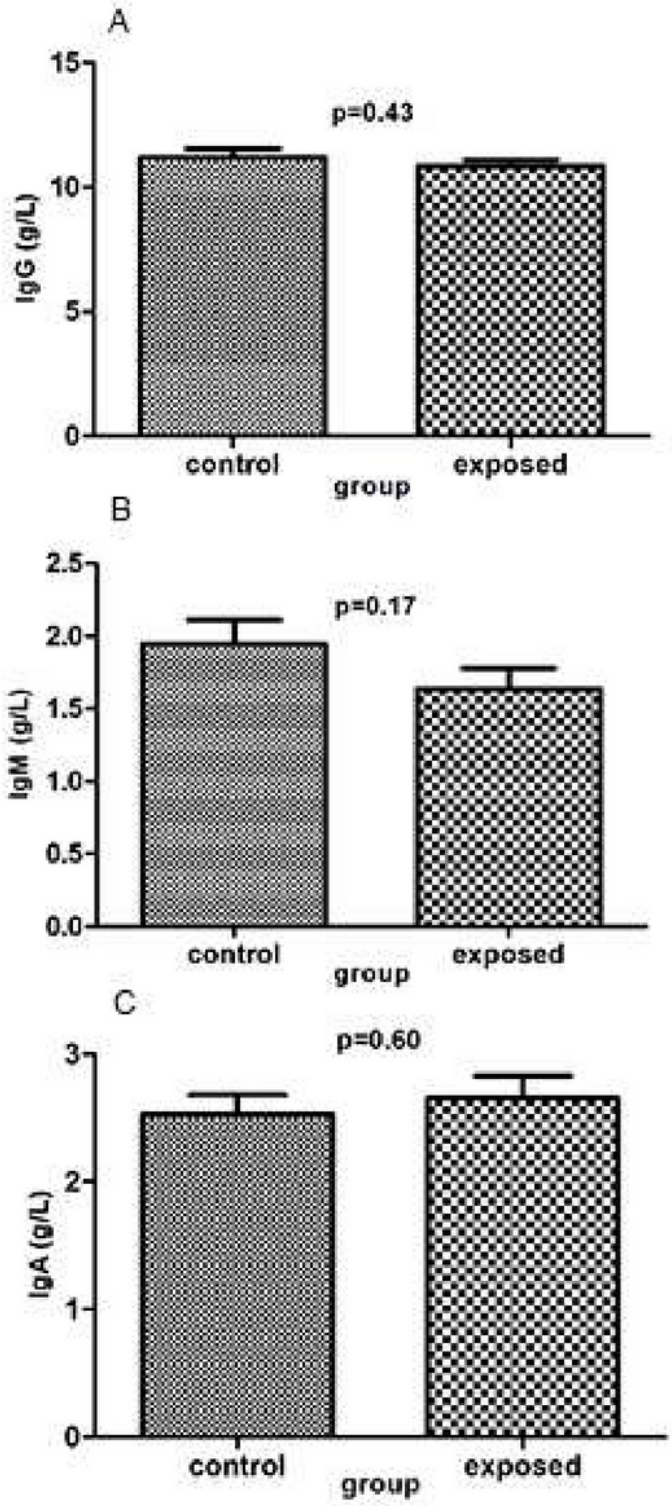

Figure 6

Statistical graph of immunoglobulin quantification by immunoturbidimetry. A, B and C represent quantification of Ig A, IgM and IgG respectively.