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Original research

The effect of N2O gas on serum cobalamin and homocysteine in patients undergoing orthopedic surgery

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Abstract

Nitrous oxide (N_2O) is an inhaled anesthetic drug that uses during surgery and recently proposed as a treatment for mood disorder in a research setting. Exposure to N2O during surgery can cause hematopoietic and neural side effects. The purpose of this study was to the designation of serum cobalamin, homocysteine and CBC factors changes after exposure to nitrous oxide during orthopedic surgery. This study has been done on 30 patients undergoing orthopedic operations from 2016 until 2018. All patients are scheduled for orthopedic surgery. All patients received nitrous oxide during their anesthesia period. Blood samples were taken from patients 48 hours before surgery and 24 hours after surgery. All samples froze at -20°c immediately after collecting. Then samples transferred to the university laboratory with regards to keeping a cold chain for analysis. Paired T-test and Wilcoxon test were used for comparing vitamin B12, homocysteine, and CBC parameters. Nitrous oxide resulted in marked vitamin B12 and homocysteine level change. Mean concentration of vitamin B12 reduced after exposure to N2O. This difference was significant when analyzed using paired sample T-test (p=0.0001). homocysteine concentration means decreased after exposure to N2O and this difference was statistically significant (p=0.0001). Nitrous oxide will lead to a decrease in vitamin B12 and an increase in homocysteine and mean corpuscular volume in the early postoperative period.

Keywords: Analgesic, Homocysteine, Nitrous oxide, Vitamin B12

1. Introduction

Nitrous oxide (N2O) is an inhaled anesthetic drug that uses during surgery and recently proposed as a treatment for mood disorder in a research setting. Exposure to N2O during surgery can cause hematopoietic and neural side effects [1-3]. Even exposure to nitrous oxide can induce ischemic stroke, aortic arch thrombosis, cortical vein thrombosis [4-6]. Vitamin B12 plays a major role in the functioning of the central nervous system of all ages. Its deficiency can be accompanied by disorders of the brain, the optic nerve, the spinal cord, and the peripheral nervous system [7]. Vitamin B12 deficiency is common in both advanced and unprocessed countries [8] Many factors, such as plant-based diet, malnutrition, congenital maladaptation, post-operative maladaptation, anti-neoplastic, and other digestive diseases (ileum cut, over-bacterial growth, and inflammatory bowel disease) can cause vitamin B12 deficiency [9]. Plasma homocysteine is elevated after exposure to N2O and may have a role in nitrous oxide side effects including pregnancy complications. In

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several patients whose plasma homocysteine levels were compared after surgery, they all had vitamin B12 deficiency. So Plasma homocysteine can be a good parameter for measuring folate-related disorders of the metabolism of cobalamin[10, 11]. In a study on 12 patients undergoing surgery, after surgery, the levels of folate and homocysteine increased and methionine was decreased. In some of these patients, even the suffering of cases has not returned to normal one week later, and it is worth noting that by determining the level of homocysteine, the effect of N2O on the deactivation of vitamin B12 can be estimated [12, 13]. So it is known that chronic exposure to nitrous oxide may have an impact on vitamin B12 and homocysteine but the effect of acute nitrous oxide exposure on vitamin B12 and homocysteine level and its impact on CBC parameters in the early postoperative period is unknown in orthopedic surgery patients. On the other hand, recent studies mostly explore the effect of recreational use of nitrous oxide but we studied acute single exposure to nitrous oxide. The purpose of this study was to the designation of serum cobalamin, homocysteine and CBC factors changes after exposure to nitrous oxide during orthopedic surgery.

2. Materials and Methods

2.1 Study design

This study has been done on 12 patients undergoing orthopedic operations from 2016 until 2018. The inclusion criteria were all patients undergoing orthopedic procedures with ages between 20 to 60 years. Exclusion criteria were patients with abnormal CBC, low level of vitamin B12 or high level of homocysteine before exposure to nitrous oxide, N2O exposure less than 120 minutes, cardiac or renal disease, pregnant or lactating women, middle ear obstruction, high intracranial pressure, and pneumothorax and patients using vitamin B12 supplement. Age, weight, height, and sex of patients were recorded. The whole procedure and aims of the study were explained to patients and written consent was obtained. This study was approved by the ethics committee of Guilan University of Medical Sciences with the approval code IR.GUMS.REC.1395.102.

2.2 Sampling and procedure

Up to 48 hours before surgery blood samples were collected from patients in two tubes. One tube contained EDTA and one without anticoagulant. Blood with anticoagulant used to measure CBC parameters and clot samples used to measure homocysteine and vitamin B12 level. Patients anesthetized by attending anesthesiologist using fentanyl 1-3 mic.kg-1, midazolam 1 mg, lidocaine 1 mg.kg-1, propofol 1.5-2.5 mg.kg-1 and atracurium 0.6 mg.kg-1 then after 3-minute patients intubated using cuffed tracheal tube. Patients' lungs are ventilated by 50 percent nitrous oxide and 50 percent oxygen. Anesthesia maintained by isoflurane 1% and muscle paralysis provided with atracurium besylate intermittent doses. If any episode of hypoxemia occurred nitrous oxide exposure ceased and the patient was excluded from the study. At the end of surgery patients ventilated with 100% oxygen and all anesthetics ceased. After returning of patient's spontaneous respiration remained effect of muscle relaxants reversed using neostigmine 0.04 mg/kg and atropine 0.02 mg/kg. After returning of patients' consciousness and adequacy of minute ventilation patients extubated and transferred to the post-anesthesia care unit (PACU). After the entrance of patients to PACU Up to 24 hours after surgery, we recollect blood samples from patients. All samples froze at -20°c immediately after collecting. then samples transferred to the university laboratory with regards to keeping a cold chain for analysis.

All samples were analyzed in the paramedical sciences school's laboratory. The serum was separated by centrifugation. Life Span BioScience quantitative competitive ELISA kit (Cat: LS-F27312) applied for determining the levels of serum Vitamin B12. Detection methods were colorimetric at 450 nm (TMB) with the range of 5-1000 pmol/L. The sensitivity of the kit was less than 1 pmol/L (Intra-Assay CV (<9%); Inter-Assay CV (<10%)). Both standard curves and samples ran in duplicate.

2.3 Statistical analysis

SPSS software version 16 was used for statistical analysis. Paired T-test and Wilcoxon test were used for comparing vitamin B12, homocysteine, and CBC parameters. A P-value less than 0.05 is considered significant.

3. Results

Among patients, there were 6 females and 6 males. The patient's age was between 20 to 67. The mean age of patients was 47.25 year. Nitrous oxide

exposure time was between 120 minutes and 420 minutes. The mean nitrous oxide exposure time was 231.83 min and the standard deviation was 84.48. Distribution of nitrous oxide exposure time was normal due to the Kolmogorov Smirnov test result.

After analyzing data using the Kolmogorov Smirnov test it was revealed that data distribution was normal for CBC parameters including white blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, homocysteine, and vitamin b12 before and after exposure to nitrous oxide.

Paired sample T-test was used to compare vitamin B12, homocysteine, white blood cell count, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration before and after exposure to nitrous oxide. The mean concentration of vitamin B12 before exposure to N2O was 527.75 (48.16) pg.ml^{-1} and reduced to 139.17 (21.20) pg.ml^{-1} after exposure to N2O. The mean of differences with a 95 percent confidence interval was between 357.85 and 419.30. This difference was significant when analyzed using paired sample T-test ($p<0.05$). The effect size for this difference is -8.03. Changes in vitamin B12 concentration showed in Figure 1.

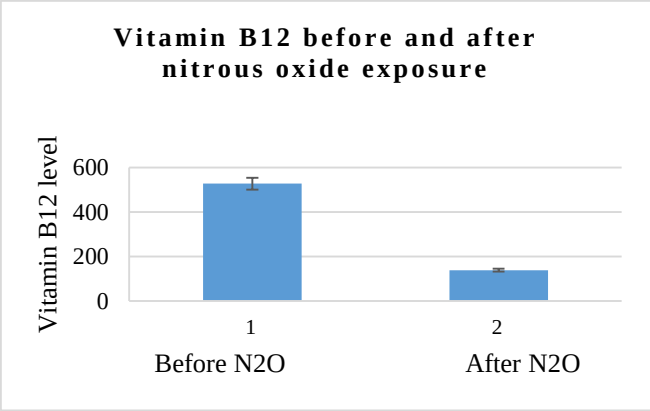

Figure1. Vitamin B12 level before and after exposure to N2o

Homocysteine concentration mean was 10.48 (0.68) µmol. l^{-1} before and 19.94(1.27) µmol. l^{-1} after exposure to N2O and this difference was statistically significant ($p<0.05$). The effect size for the difference in homocysteine before and after exposure to nitrous oxide is 6.47. The mean of differences in homocysteine level before and after exposure to nitrous oxide with a 95 percent of the confidence interval was between 8.52 and 10.39. Homocysteine concentration changes before and after surgery showed in Figure 2. Paired T-test analysis results are described in Table 1.

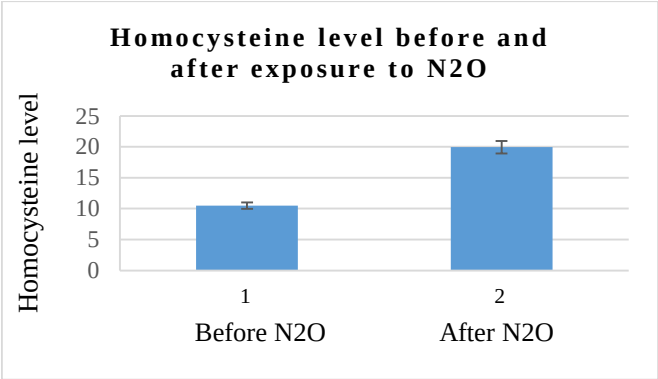

Figure 2. Homocysteine level before and after exposure to N2O

Wilcoxon test was used to compare CBC parameters including red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and platelet before and after exposure to N2O.

There was a statistically significant difference for RBC and MCV before and after nitrous oxide exposure ($p<0.05$) MCH and platelet count did not change before and after surgery significantly. These data are depicted in Table 2.

4. Discussion

This study showed that nitrous oxide exposure during orthopedic surgery can affect vitamin B12 and homocysteine levels in the early postoperative period. The mean concentration of vitamin B12 before exposure to N2O was 527.75 (48.16) pg.ml-1 and reduced to 139.17 (21.20) pg.ml-1 after exposure to N2O. Homocysteine concentration mean decreased after exposure to N2O and this difference was statistically significant ($p<0.05$).

Nitrous oxide prescribed to patients for more than a century and has favorable characteristics including low tissue solubility, analgesia, no or minimal cardiovascular depressant effect, and low cost. Despite these advantages, nitrous oxide may cause nausea and vomiting, bowel distension, act as a greenhouse gas, and vitamin B12 deficiency.

The most important clinical symptom after exposure to N2O is a deficiency in vitamin B12 level. N2O oxidizes vitamin B12 and converts it from active to an inactive form [14]. Moreover, applies its harmful

Table 1. Mean, standard deviation, and differences of measured parameters before and after exposure to nitrous oxide

Variable	Mean (SD) Before exposure	Mean (SD) After exposure	P-value*
Vitamin B12	527.75 (48.16)	139.17 (21.20)	<0.001
Homocysteine	10.48 (0.68)	19.94 (1.27)	<0.001
White blood cell count	9766.70 (3802.47)	12292.0 (4522.96)	0.03
Hemoglobin	11.51 (1.2)	10.63 (1.38)	0.02
Hematocrit	34.10 (3.07)	31.85 (3.60)	0.04
Mean corpuscular hemoglobin concentration	33.72 (1.88)	33.30 (1.77)	0.12

*Paired sample T-test

Table 2. Mean and standard deviation of RBC, MCV, MCH, and platelet count

Variable	Mean (SD) Before exposure	Mean (SD) After exposure	Mean rank		z	P-value
Red blood cell count	4.04 (0.60)	3.73 (0.65)	Negative rank	6.60	-2.12	0.03
			Positive rank	6.00		
Mean corpuscular volume	85.25 (9.85)	86.55 (9.69)	Negative rank	5.00	-2.27	0.02
			Positive rank	6.80		
Mean corpuscular hemoglobin	28.80 (4.01)	28.90 (4.06)	Negative rank	4.75	-0.40	0.68
			Positive rank	7.50		
Platelet count	247330.0 (106491.0)	260250.0 (134538.0)	Negative rank	6.20	-0.62	0.53
			Positive rank	6.71		

effects with the oxidation of cobalt ion in cobalamin and its conversion from the capacity (+1) to (+ 3) irreversibly. The oxidation of cobalt ion by nitrous oxide prevents methylcobalamin as a co-enzyme in the production of methionine and subsequently S-adenosylmethionine, which is essential for the methylation of phospholipids in the myelin sheath and the result is reduced myelin building [15, 16].

In our study, we aimed to evaluate nitrous oxide exposure effect on vitamin B12, homocysteine, and CBC factors in orthopedic surgery patients. We found that exposure to nitrous oxide even for short time (between 120 to 420 minutes) will decrease vitamin B12 level significantly. There is a similar result in the study of Glinj et al., Lundin et al., and Noh et al. [17-19]; however, controversy was seen in a previous study [20]. Vitamin B12 may be affected during anesthesia using nitrous oxide which may lead to granulocytopenia, megaloblastic anemia, thrombocytopenia, leaukopenia, and bone marrow suppression. When we evaluate the correlation between age and the effect of nitrous oxide on homocysteine level, we found a significant negative correlation between these parameters. Previous study revealed that nitrous oxide would increase homocysteine in children [21]. Our study cleared that increase in age increase this effect. On the other hand, powers et al, revealed that nitrous oxide would increase homocysteine in old rats but their study there was on rats and we evaluate nitrous oxide effect on humans [22]. Nitrous oxide binds to vitamin B12 and oxidizes it irreversibly and therefore decreases vitamin B12 level. Most studies emphasize on duration and concentration of nitrous oxide exposure as a determinant factor in vitamin B12's destructive effect [23]. But we found that time has no effect on nitrous toxicity which is consistent with Patel et al., study [24]. Studies revealed that vitamin B12 deficiency induced by nitrous oxide is severe and according to the latest studies vitamin B12 level lower than 200 pg/ml considered deficient which is consistent with our study results [25, 26]. The mean vitamin B12 value after exposure to nitrous oxide was 169.00 which is considered deficient. Based on our finding it seems to short-time exposure to nitrous oxide will inactive vitamin B12 and decrease its blood level which may

lead to numerous complications including subacute combined degeneration of the spinal cord and even acute psychosis [27]. It is proposed that nitrous oxide used with more cautions in patients with known vitamin B12 deficiency diagnosed based on neurologic symptoms or laboratory investigations.

We measured homocysteine level in addition to vitamin B12. Results showed that there was increased homocysteine level in all cases exposed to nitrous oxide and there was a statistically significant difference in homocysteine level before and after exposure to nitrous oxide. Our finding is consistent with Lan et al., Myles et al., and Nagele et al., studies [28-30]. Lan et al. found that exposure to nitrous oxide will increase homocysteine level and patients with increased homocysteine level experienced neurologic dysfunctions including numbness, paresthesia, weakness, ataxia, impaired cognition, and other neurologic symptoms [30]. Pichardo et al., found that exposure to nitrous oxide for more than two hours in pediatric patients will increase homocysteine levels [21]. But in our study, we found that there is no relationship between nitrous oxide exposure time and homocysteine level. In our study, neither vitamin B12 level nor homocysteine did not get impressed by nitrous oxide exposure time.

It was interesting that in the acute phase of nitrous oxide exposure time patients experienced an increase in mean corpuscular volume and this increase was statistically significant. Our finding was against Duma et al. [31], this difference may be due to the different age groups studied. It is known that lack of vitamin B12 may lead to megaloblastic anemia but the pace of this event was too much rapid. On the other hand, there was a significant reduction in hemoglobin in our patients and, predictably, this reduction may lead to microcytic anemia which probably attenuates the effect of nitrous oxide on mean corpuscular volume. Intraoperative fluid therapy had been done with isotonic solutions thus increase in MCV can't be related to fluid therapy. We don't know whether this effect persists hours or days after surgery in adults and elderly populations or not. Also, we don't know if patients get nitrous oxide for a long-time but without bleeding surgeries how would be MCV impacted. We propose future studies explore the presence and severity of this effect in days after exposure to nitrous oxide and surgeries without or with minimal bleeding.

According to the vitamin B12 metabolism cycle, it is logical that decreased vitamin B12 leads to increased homocysteine. Vitamin B12 is a key factor in converting homocysteine to methionine. Thus when there is a lack of vitamin B12 or there is a nonfunctional form of this vitamin it will result in increased homocysteine [32]. Homocysteine is a sulfur-containing amino acid that participates in methionine metabolism. High homocysteine level is an independent risk factor for cardiovascular disease including coronary artery disease, cerebrovascular accident and can lead to high blood pressure and left ventricular hypertrophy [33-35]. Even a five micromoles increase in homocysteine level more than normal amounts can increase the risk of cardiovascular disease up to 20 percent. Homocysteine has many effects on all three layers of the arterial wall including endothelium, tunica media, and tunica adventitia. Homocysteine induces inflammation, apoptosis, atherosclerosis plaque formation, smooth muscle proliferation, collagen formation, and elastin degeneration in the arterial wall [35]. So, it can be concluded that homocysteine can act as both indicators of low vitamin B12 function and a predictor of cardiovascular events. So, it can be assumed that exposure to nitrous oxide may put patients at risk of cardiovascular events. A direct relationship between nitrous oxide use, homocysteine level, and cardiovascular events needs further investigations. Some case reports showed myelin sheath defects due to nitrous oxide recreational use [30]. Vitamin B12 is crucial in the central nervous system for synthase and maintenance of myelin sheath. It is clarified that lack of vitamin B12 can lead to subacute combined degeneration which is characterized by a defect in myelin sheath in the lateral and dorsal part of the cervical and thoracic spine, brain white matter demyelination, and peripheral neuropathy [24]. When we explored CBC parameters, we found a statistical difference in white blood cells, red blood cells, hemoglobin, hematocrit, and mean corpuscular volume before and after surgery. Other parameters including mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and platelet count did not change statistically before and after surgery and exposure to nitrous oxide. Based on our finding it is rational to consider measurement of vitamin B12 and homocysteine after surgery in patients susceptible to vitamin B12 deficiency-like old

patients, vegans, and patients with malabsorptive diseases are to undergo nitrous oxide anesthesia. It may help in the reduction of cardiovascular, neurologic, and hematologic complications. As a limitation, we did not assess neurologic and cardiovascular complications due to vitamin B12 deficiency and homocysteine increase. We propose a study on these complications in future studies.

The most important limitation of this study was a small number of orthopedic patients anesthetized with nitrous oxide, which can

Authors' contributions

MKH and MA designed the study, AA, MKH and AB performed the study. MA and MKH do analysis and interpreting the data. All authors discussed the results and participated in the final version of the manuscript.

Conflict of interests

Authors declare that there is no conflict of interest.

Ethical declarations

The whole procedure and aims of the study were explained to patients and written consent was obtained. This study design was approved by the ethics committee of Guilan University of Medical Sciences with the approval code IR.GUMS.REC.1395.102.

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References

1. Ilniczky S, Jelencsik I, Kenez J, Szirmai I. MR findings in subacute combined degeneration of the spinal cord caused by nitrous oxide anaesthesia-two cases. Eur J Neurol. 2002; 9(1):101-4.
2. Keddie S, Adams A, Kelso AR, Turner B, Schmierer K, Gnanapavan S, et al. No laughing matter: subacute degeneration of the spinal cord due to nitrous oxide inhalation. J Neurol. 2018; 265(5):1089-95.
3. Nagele P, Zorumski CF, Conway C. Exploring Nitrous Oxide as Treatment for Mood Disorders: Basic Concepts. J Clin Psychopharmacol. 2018; 38(2):144.
4. Bajaj D, Agrawal A, Gupta S, Bajaj S. Recreational nitrous oxide abuse causing ischemic stroke in a young patient: a rare case report. Cureus. 2018; 10(12).
5. Sjoerd H, Vermeulen EG, Metz R, Rijbroek A, de Vries M. Aortic arch thrombus caused by nitrous oxide abuse. J Vasc Surg Cases Innov Tech. 2018; 4(2):80-2.
6. Liu M, Zhang J, Bu B. Isolated cortical vein thrombosis after nitrous oxide use in a young woman: a case report. BMC Neurol. 2020; 20(1):1-5.
7. Naidich MJ, Ho SU. Case 87: Subacute combined degeneration. Radiology. 2005; 237(1):101-5.
8. Lim CT. Neuroimaging in postinfectious demyelination and nutritional disorders of the central nervous system. Neuroimaging Clin N Am. 2011; 21(4):843-58.
9. Oh R, Brown DL. Vitamin B12 deficiency. Am Fam Physician. 2003; 67(5):979-86.
10. Stabler SP, Marcell PD, Podell ER, Allen RH, Savage DG, Lindenbaum J. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. J Clin Investig. 1988; 81(2):466-74.
11. Gaiday AN, Tussupkaliyev AB, Bermagambetova SK, Zhumagulova SS, Sarsembayeva LK, Dossimbetova MB, et al. Effect of homocysteine on pregnancy: a systematic review. Chem Biol Interact. 2018; 293:70-6.
12. Amess J, Rees G, Burman J, Nancekievill D, Mollin D. Megaloblastic haemopoiesis in patients receiving nitrous oxide. The Lancet. 1978; 312(8085):339-42.
13. Skacel P, Hewlett A, Lewis J, Lumb M, Nunn J, Chanarin I. Studies on the haemopoietic toxicity of nitrous oxide in man. Br J Haematol. 1983; 53(2):189-200.
14. Drummond JT, Matthews RG. Nitrous oxide inactivation of cobalamin-dependent methionine synthase from Escherichia coli: characterization of the damage to the enzyme and prosthetic group. Biochem. 1994; 33(12):3742-50.
15. Yagiela JA. Health hazards and nitrous oxide: a time for reappraisal. Anesth Prog. 1991; 38(1):1.
16. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. Neurology. 1995; 45(8):1435-40.
17. Glijn N, van der Linde D, Ertekin E, Van Burg P, Grimbergen YA, Libourel E. Is nitrous oxide really that joyful. Neth J Med. 2017; 75(7):304-6.
18. Lundin MS, Cherian J, Andrew MN, Tikaria R. One month of nitrous oxide abuse causing acute vitamin B 12 deficiency with severe neuropsychiatric symptoms. BMJ Case Rep. 2019; 12(2):bcr-2018-228001.
19. Noh T, Osman G, Chedid M, Hefzy H. Nitrous oxide-induced demyelination: Clinical presentation, diagnosis and treatment recommendations. J Neurol Sci. 2020:116817.
20. Desprairies C, Imbard A, Koehl B, Lorrot M, Gaschignard J, Sommet J, et al. Nitrous oxide and vitamin B12 in sickle cell disease: Not a laughing situation. Mol Genet Metab Rep. 2020; 23:100579.
21. Pichardo D, Luginbuehl IA, Shakur Y, Wales PW, El-Sohemy A, O'Connor DL. Effect of nitrous oxide exposure during surgery on the homocysteine concentrations of children. Anesthesiology. 2012; 117(1):15-21.
22. Powers RW, Majors AK, Lykins DL, Sims CJ, Lain KY, Roberts JM. Plasma homocysteine and malondialdehyde are correlated in an age-and gender-specific manner. Metab Clin Exp. 2002; 51(11):1433-8.
23. Chaugny C, Simon J, Collin-Masson H, De Beauchene M, Cabral D, Fagniez O, et al. Vitamin B12 deficiency due to nitrous oxide use: unrecognized cause of combined spinal cord degeneration. Rev Med Interne. 2013; 35(5):328-32.

24. Patel KK, Munne JCM, Gunness VRN, Hersey D, Alshafai N, Sciubba D, et al. Subacute combined degeneration of the spinal cord following nitrous oxide anesthesia: a systematic review of cases. Clin Neurol Neurosurg. 2018; 173:163-8.
25. Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood. 2017; 129(19):2603-11.
26. Ankar A, Kumar A. Vitamin B12 deficiency. StatPearls [Internet]: StatPearls Publishing; 2022.
27. Wong SL, Harrison R, Mattman A, Hsiung G-YR. Nitrous oxide (N 2 O)-induced acute psychosis. Can J Neurol Sci. 2014; 41(5):672-4.
28. Nagele P, Tallchief D, Blood J, Sharma A, Kharasch ED. Nitrous oxide anesthesia and plasma homocysteine in adolescents. Anesth Analg. 2011; 113(4):843.
29. Myles PS, Chan MT, Kaye DM, McIlroy DR, Lau C-W, Symons JA, et al. Effect of nitrous oxide anesthesia on plasma homocysteine and endothelial function. Anesthesiology. 2008; 109(4):657-63.
30. Lan S-Y, Kuo C-Y, Chou C-C, Kong S-S, Hung P-C, Tsai H-Y, et al. Recreational nitrous oxide abuse related subacute combined degeneration of the spinal cord in adolescents–a case series and literature review. Brain Dev. 2019; 41(5):428-35.
31. Duma A, Cartmill C, Blood J, Sharma A, Kharasch E, Nagele P. The hematological effects of nitrous oxide anesthesia in pediatric patients. Anesth Analg. 2015; 120(6):1325.
32. Hathout L, El-Saden S. Nitrous oxide-induced B12 deficiency myelopathy: perspectives on the clinical biochemistry of vitamin B12. J Neurol Sci. 2011; 301(1-2):1-8.
33. Wang L, Niu H, Zhang J. Homocysteine induces mitochondrial dysfunction and oxidative stress in myocardial ischemia/reperfusion injury through stimulating ROS production and the ERK1/2 signaling pathway. Exp Ther Med. 2020; 20(2):938-44.
34. Kozakova M, Morizzo C, Penno G, Shore AC, Nilsson J, Palombo C. Plasma Homocysteine and Cardiovascular Organ Damage in a Population with a High Prevalence of Risk Factors. J Clin Endocrinol Metab. 2020; 105(8):dgaa289.
35. Balint B, Jepchumba VK, Guéant J-L, Guéant-Rodriguez R-M. Mechanisms of homocysteine-induced damage to the endothelial, medial and adventitial layers of the arterial wall. Biochimie. 2020. 173:100-106.