



Complications caused by nitrous oxide in dental sedation

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The first clinical application of nitrous oxide (N₂O) was in 1844, by an American dentist named Horace Wells who used it to control pain during tooth extraction. Since then, N₂O has shared a 170-year history with modern dental anesthesia. N₂O, an odorless and colorless gas, is very appealing as a sedative owing to its anxiolytic, analgesic, and amnesic properties, rapid onset and recovery, and, in particular, needle-free application. Numerous studies have reported that N₂O can be used safely and effectively as a procedural sedation and analgesia (PSA) agent. However, N₂O can lead to the irreversible inactivation of vitamin B₁₂, which is essential for humans; although rare, this can be fatal in some patients.

Keywords: Conscious Sedation; Nitrous Oxide; Vitamin B₁₂ Deficiency.



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INTRODUCTION

Many patients experience dental fear and anxiety (DFA), which imposes a significant level of stress in dentists who must treat such patients [1-3]. While there are various non-pharmacological methods for dealing with DFA, these methods may not be effective in patients with severe DFA; therefore, pharmacological approaches including sedation and general anesthesia (GA) may be unavoidable in treating some patients [4]. Although there is very little difference in the prevalence of DFA between adults and children [1,2], adults are able to avoid their DFA by canceling or delaying their own dental appointments, whereas children often do not have such options. Moreover, children are often unable to repress their expression of fear, which may manifest as excessive

crying and/or physical struggle. These reasons led to the early adoption of sedation in pediatric dentistry. According to a survey of the members of International Association of Paediatric Dentistry (IAPD) and European Academy of Paediatric Dentistry (EAPD), the pharmacological method most often used for behavioral control was GA (52%), followed by N₂O-only sedation (46%) and oral sedation (44%) [5]. The objective of this review was to investigate the properties of N₂O as a PSA agent and identify the adverse events (AEs) associated with N₂O.

N₂O FOR PROCEDURAL SEDATION AND ANALGESIA

Since its initial introduction in 1844 by an American dentist named Horace Wells for pain control during tooth

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extraction, N₂O has been widely used in dentistry to control the pain and distress in patients [6]. N₂O is an odorless and colorless gas with anxiolytic, analgesic, and amnesic properties, along with rapid onset and recovery, which represent the ideal characteristics of a sedative [6,7]. Moreover, as a major advantage of using N₂O is the mitigation of needle phobia [7], it is therefore commonly to achieve PSA in pediatric patients. In particular, N₂O is used for simple venipuncture [8], with Pasarón and colleagues [9] reporting that 98% of patients who underwent PSA by N₂O did not even remember the injection. In other words, patients who are sedated by the inhalation of N₂O through a mask are not only very receptive to being injected by a needle owing to the anxiolytic and analgesic effects of N₂O (often being completely unaware of needling itself), but they may also not remember the injection. Undoubtedly, these characteristics of N₂O make it a very appealing sedative for patients who have a significant phobia of needles. However, PSA with N₂O alone may not provide a sufficient analgesic effect in procedures that can cause severe pain, such as fracture reduction or foreign body removal [10].

In many countries, including the US, Australia, and France, there have been reports on the safe use of N₂O as a PSA agent, where it is also used in diverse areas, including dentistry, radiology, orthopedics, and the emergency department (ED) [9,11-15]. Unlike Korea, N₂O can be used in the US and France without a dentist, doctor, or anesthesiologist present [11,14,16]; naturally, nurses who can perform N₂O sedation are registered nurses who have completed N₂O certification courses [17].

There have been many studies in France on the safety and efficacy of N₂O as a PSA agent [14,15,18,19]. However, because the use of N₂O in France is based on a 50% N₂O/O₂ premix one-bottle system [14], those

studies from France were excluded in this review owing to the potential for slight differences to the two-bottle system used in Korea.

There is a risk of diffusion hypoxia after cessation of N₂O [20], which may be minimized by using O₂ together with N₂O where 100% O₂ is supplied after cessation of N₂O [21]. Moreover, the N₂O inhalation sedation unit most widely used today (MDM, Matrx, NY, USA) has a minimum O₂ concentration setting of 25%-30% and has an O₂ fail-safe system, in which the unit automatically shuts off the supply of N₂O when the oxygen pressure drops below a certain preset level [22]. Although there is no specific formula or a general rule for how long 100% O₂ should be supplied after cessation of N₂O, longer sedation times typically requires a longer time to remove the sedative effect [22]. Some studies have reported no difference in recovery under room air versus 100% O₂ supply [23]. However, a 2010 study by Zier et al. [24] reported that three out of six atypical AEs were associated with apnea/O₂ desaturation after recovery under room air when patients were supplied with 100% O₂ for 2-3 minutes after cessation of N₂O. Meanwhile, Malamed [22] reported that 100% O₂ should be supplied for at least 3-5 minutes for the complete elimination of N₂O from the body, after which the patient should be re-evaluated to determine whether additional 100% O₂ should be supplied.

The most common AE associated with using PSA with N₂O is vomiting [9,12,13,24]. Burnweit et al. [25] reported that preoperative fasting is a cause of nausea and vomiting. In a retrospective review of 12 years of cases from a single institution, the rate of vomiting among patients who were asked to eat something light during 2 hours before the procedure was 0.7%, which was lower than the vomiting rates found in other studies (Table 1)

Table 1. Study of adverse events of nitrous oxide/oxygen procedural sedation and analgesia

| Study | Country | Total number of patients | Nitrous oxide: oxygen ratio | Serious adverse events, % | Minor adverse events, % | Vomiting, % |
|---------------------------|-----------|--------------------------|-----------------------------|---------------------------|-------------------------|-------------|
| Babl et al. (2008) [13] | Australia | 762 | Up to 70:30 | 0.3 | 8.3 | 5.7 |
| Zier & Liu (2011) [12] | USA | 7,802 | Up to 70:30 | 0.14 | 5.0 | 2.2 |
| Pasarón et al. (2015) [9] | USA | 1,058 | Up to 60:40 | 0 | 1.8 | 0.7 |

Table 2. Description of serious adverse events during nitrous oxide/oxygen procedural sedation and analgesia

| Study | Patient's age | Percentage of nitrous oxide | Description of serious adverse events | Notes |
|-------------------------|---------------|---|--|--|
| Babl et al. (2015) [26] | 16 months | 70% | Laryngospasm | |
| Babl et al. (2008) [13] | 11 years | 70% | Stabbing central chest pain | 2.5 mg Morphine sulfate IV 1 hour before PSA |
| Zier et al. (2010) [24] | 12 years | 70% | Desaturation | Trisomy 21 |
| | 2 years | 50% | Apnea >15 seconds (on return to room air) | After cessation of N ₂ O, 100% O ₂ 3 min |
| | 16 months | 65-70% | Desaturation to 89% (on return to room air) | After cessation of N ₂ O, 100% O ₂ 2 min |
| | 3 years | 70% | Unresponsive/desaturation to 89% (On return to room air) | After cessation of N ₂ O, 100% O ₂ 3 min |
| | 2 months | 70% | Stridor | |
| Zier et al. (2010) [28] | 12 months | 1 st : 70% for 4 min 2 nd : 50% for 8 min 3 rd : 65% | Tonic-clonic seizure for 3 min just after the 3 rd administration | |
| | 2 years | 60% 9 min | Tonic-clonic seizure | One probable nonfebrile seizure history After cessation of N ₂ O, during administration of 100% O ₂ |
| | 17 months | 70% 4 min | Tonic-clonic seizure | Two febrile and one nonfebrile seizure history Familial history for febrile seizures |

IV: Intravenous injection, PSA: Procedural sedation and analgesia, N₂O: nitrous oxide, O₂: Oxygen, min: minutes.

[9]. In the study by Babl et al. [13], which required at least 2 hours of preoperative fasting, 5.7% presented with vomiting. In Zier & Liu's study 2.2% presented with vomiting [12]. In Zier & Liu's study, four hours of preoperative fasting was required for patient who underwent N₂O procedure during the first 4 months of the study; a light meal during the 4 hours before the procedure was advised for the 6 months; and no fasting-related requirement was given for the rest of the study period as the interim analysis on the first two periods revealed no difference in AE rates. In addition, this study also reported that frequency of minor AEs (MAEs) increased with longer treatment time or deeper sedation [9]. Meanwhile, Zier and Liu reported that the frequency of AEs increased with a longer duration of N₂O supply [12].

Recently, laryngospasm was reported after PSA with only N₂O/O₂ [26]. As laryngospasm does not occur under minimal or moderate sedation, only when the patient is under deep sedation or light GA [22], this indicated that 70% N₂O induced deep sedation, close to GA, for the patients in this case. Because drug reactions vary greatly between patients, N₂O (1 MAC = 105-107, MAC: minimal alveolar concentration) may induce deep sedation

in some patients, sufficient to cause laryngospasm [27]. Moreover, because dental treatments regions coincided with upper airway space, there is a high risk of airway irritation during dental treatment. Thus, reactions of a patient must be carefully observed to titrate the concentration of N₂O.

In addition, other serious AEs (SAEs), including stabbing central chest pain, O₂ desaturation, apnea > 15 seconds, stridor, and tonic-clonic seizure have been reported (Table 2); these SAEs occurred when a high concentration of N₂O, from over 50% to nearly 70%, was used [13,24,26,28]. The 2011 survey of 311 members of IAPD and EAPD reported one case of N₂O-related death, but did not give specific details [5].

VITAMIN B₁₂ INACTIVATION BY N₂O

N₂O may cause irreversible inactivation of vitamin B₁₂ [29], an essential nutrient that acts as a cofactor in the folate and methionine cycles in humans [30]. However, because the human body is unable to synthesize vitamin B₁₂, it must be obtained through the consumption of foods of animal origin [31].

Vitamin B₁₂ deficiency may cause megaloblastic anemia in the peripheral blood and bone marrow, subacute combined degeneration (SCD) of the spinal cord, polyneuropathy, optic nerve injury, glossitis, dementia, thrombosis, and/or infertility [31-34]. In children, the possibility of Vitamin B₁₂ deficiency should be carefully monitored as it can impair the development of the brain and the overall growth, which may lead to permanent disabilities [35,36].

Vitamin B₁₂ deficiency may be caused by various factors, including genetic factors such as 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency, impaired vitamin B₁₂ absorption (observed in pernicious anemia, inflammatory bowel diseases such as Crohn's disease, a history of partial/total gastrectomy or ileal resection, gastric subacidity, and the use of metformin), insufficient dietary consumption of vitamin B₁₂ (in vegetarians/vegans, or infants breastfed by mothers with vitamin B₁₂ deficiency), as well as repeated occupational

or recreational exposure to N₂O [31,37-39].

However, when vitamin B₁₂ deficiency is subclinical, such patients may appear to be healthy and consequently classified as ASA class I without any suspicion; indeed, they may even have a previous history of uneventful N₂O anesthesia or sedation [40]. Caution should be taken as the use of N₂O on such patients may result in fatal outcomes [40].

The first case of hematological changes as a result of prolonged N₂O use was a report by Goilmsen in 1955 [41]. At the time of the report, prolonged N₂O use was not identified as the cause; this was subsequently determined by Lassen et al. in 1956 [42]. In this study, GA, which included the use of N₂O, for the treatment of tetanus, was performed over several days and severe bone marrow depression was found 4-17 days after the use of N₂O [42]. Since then, there have been numerous case reports of N₂O toxicity (Table 3) [32,37,40,43-48]. Symptoms appeared from after 2 days to even after 2

Table 3. Summary of case studies on nitrous oxide toxicity

| Study | Patient's age | Concentration and duration of exposure to N ₂ O | Time of onset of symptoms | History or undiagnosed disease | Treatment | Consequences |
|----------------------------------|---------------|---|--|--|---|--|
| Lassen et al. (1956) [42] | 10 years | 14 days | 14 th day | — | — | — |
| | 11 years | 12 days | 6-10 th day | — | — | — |
| | 15 years | 17-18 days | 17-18 th day | — | — | Mortality (on 29 th day) |
| | 53 years | 16 days | 16 th day | — | — | Mortality (on 16 th day) |
| Koblin and Biebuyck (1986) [43] | 25 years | 1 st : 90 min, 1980, Mar. 2 nd : 1982, Apr. | 2 months later | Ileal resection for Crohn's disease | Cyanocobalamin injection | Reversible |
| | 58 years | 90 min | 6 weeks later | Pernicious anemia | Cyanocobalamin injection | Reversible |
| Hadzic et al. (1995) [44] | 47 years | 70% for 8 hours | 6 weeks later | Pernicious anemia | Cobalamin injection | Reversible |
| Rosener and Dichgans (1996) [45] | 50 years | 66% for 2 hours | 4 weeks later | Vegetarian diet | Cyanocobalamin injection | Reversible |
| McNeely et al. (2000) [37] | 6 months | — | 2 weeks later | Breast feeding vegetarian mother | Vitamin B ₁₂ supplementation | Developmental delay |
| Ilniczky et al. (2002) [32] | 52 years | — | 1 week later | Macrocytic, hyperchromic anemia with decreased serum levels of vitamin B ₁₂ | IM injection of vitamin B ₁₂ | Reversible |
| | 57 years | — | 2 months later | Borderline anemia with decreased serum levels of vitamin B ₁₂ | IM injection of vitamin B ₁₂ | Reversible |
| Selzer et al. (2003) [40] | 3 months | 1 st : 60% for 45 min 2 nd : 60% for 270 min (4 days after 1 st anesthesia) | 25 days after 2 nd anesthesia | MTHFR deficiency | — | Mortality (46 days after 2 nd anesthesia) |

(Continued to the next page)

Table 3. Continued

| Study | Patient's age | Concentration and duration of exposure to N ₂ O | Time of onset of symptoms | History or undiagnosed disease | Treatment | Consequences |
|-----------------------------|---------------|--|--|--------------------------------|--|--------------|
| Lacassie et al. (2006) [46] | 52 years | 1 st : 50% for 200 min for 2 nd : 50%, 105 min (8 weeks after 1 st anesthesia) | 2 weeks after 1 st anesthesia | Polymorphism of MTHFR | Vitamin B ₁₂ and folic acid supplementation | Reversible |
| Singer et al. (2008) [47] | 27 years | — | 2 months later | Pernicious anemia | IM injection of vitamin B ₁₂ | Reversible |
| Renard et al. (2009) [48] | 46 years | — | 2 days later | Borderline anemia | IM injection of vitamin B ₁₂ | Reversible |

Min: minutes, IM: Intramuscular, MTHFR: 5,10-Methylenetetrahydrofolate Reductase.

months, with the initial symptoms including symmetric paresthesia or numbness in the limbs, which gradually spread to the trunk to cause gait unsteadiness. In most patients, the injection or oral supplements of vitamin B₁₂ may be effective to alleviate the symptoms, but sensory impairment and other sequelae may persist. In particular, N₂O toxicity may be fatal in pediatric patients who are still in a developmental stage [37,40].

Previously, reports on N₂O toxicity have been related to GA, recreational abuse, and occupational exposure [49-53]. However, the important factor was not whether N₂O was used for GA or PSA, but the degree of exposure to N₂O: that is, the concentration of N₂O used and how long and often the patient was exposed to N₂O were important [54-56]. In addition, as mentioned above, patients with diagnosed or undiagnosed vitamin B₁₂ deficiency may experience symptoms from a single exposure to N₂O. Even in those patients with no signs of vitamin B₁₂ deficiency, repeated occupational exposure or recreational abuse may place them at high-risk of N₂O toxicity.

N₂O interferes with the process of transformation from homocysteine and methionine through the inactivation of vitamin B₁₂; consequently, it causes elevation of the plasma homocysteine concentration [57]. In 2008, Nagele et al. [58] reported that exposure to 66% N₂O for over 4 hours resulted in a significant increase in postoperative plasma homocysteine concentration levels, whereas in 2013, Hakimoglu et al. [59] reported that postoperative plasma homocysteine concentration level was significantly higher when the duration of GA with 60% N₂O was > 3 hours than when it was < 3 hours. Amos et

al. [60] reported that megaloblastic bone-marrow change occurred in patients who underwent GA with N₂O for ≥ 2 hours, which was affected more by the general condition of the patient than the length of N₂O exposure. Moreover, in the deoxyuridine (dU) suppression test for the assessment of abnormalities in DNA synthesis, all 15 patients who did not undergo GA with N₂O showed normal results, whereas 39 of 42 patients who underwent GA with N₂O showed abnormal results, and this difference was detected for patients who were exposed to N₂O for a minimum of 1 hour.

It is not simple for experts to recognize and diagnose vitamin B₁₂ deficiency in a clinical setting [31]. However, since patients with vitamin B₁₂ deficiency sometimes present with neurological symptoms or anemia and as over 98% of them have increased serum methylmalonic acid and total homocysteine levels, a preoperative evaluation of these parameters may be helpful for the identification of patients at risk of N₂O toxicity.

Although there are differences in prevalence based on ethnicity, the number of patients with risk factors for N₂O toxicity is a low proportion of the total population [61]. Indeed, relevant information often goes unmentioned in contraindications for PSA with N₂O. Nevertheless, based on cases reported from time to time, this factor should not be underestimated. If screening is possible prior to PSA, the use of a suitable alternative sedative would be preferable. Moreover, if related symptoms occur after PSA with N₂O, early detection and appropriate treatment may reverse such symptoms. Therefore, it is necessary to provide an introduction and warnings about possible initial symptoms to patients who may not have been screened.

CONCLUSION

Since the introduction of clinical anesthesiology, N₂O has been used for sedation and analgesia, and it remains a popular option. The rate of SAEs after PSA with only N₂O is low (0–0.3%), with vomiting being the most common AE. Cases of laryngospasm after a high-dose of N₂O have been reported, and even a few cases of death. Furthermore, in patients who are repeatedly exposed to N₂O or have vitamin B₁₂ deficiency, various neurological symptoms may result from N₂O-induced vitamin B₁₂ inactivation. In summary, while sedation with N₂O rarely presents with SAEs, further investigation on understanding of N₂O-related AEs and their triggers may prove to be beneficial towards patients with greater risk.

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REFERENCES

1. Chanpong B, Haas DA, Locker D. Need and demand for sedation or general anesthesia in dentistry: A national survey of the canadian population. *Anesth Prog* 2005; 52: 3-11.
2. Klingberg G, Broberg AG. Dental fear/anxiety and dental behaviour management problems in children and adolescents: A review of prevalence and concomitant psychological factors. *Int J Paediatr Dent* 2007; 17: 391-406.
3. Brahm C-O, Lundgren J, Carlsson SG, Nilsson P, Corbeil J, Hägglin C. Dentists' views on fearful patients. *Problems and promises. Swed Dent J* 2012; 36: 79-89.
4. Armfield JM, Heaton L. Management of fear and anxiety in the dental clinic: A review. *Aust Dent J* 2013; 58: 390-407.
5. Wilson S, Alcaino EA. Survey on sedation in paediatric dentistry: A global perspective. *Int J Paediatr Dent* 2011; 21: 321-32.
6. Huang C, Johnson N. Nitrous oxide, from the operating room to the emergency department. *Curr Emerg Hosp Med Rep* 2016; 4: 11-8.
7. Baum VC, Willschke H, Marciniak B. Is nitrous oxide necessary in the future? *Pediatr Anesth* 2012; 22: 981-7.
8. Gerhardt RT, King KM, Wiegert RS. Inhaled nitrous oxide versus placebo as an analgesic and anxiolytic adjunct to peripheral intravenous cannulation. *Am J Emerg Med* 2001; 19: 492-4.
9. Pasaron R, Burnweit C, Zerpa J, Malvezzi L, Knight C, Shapiro T, et al. Nitrous oxide procedural sedation in non-fasting pediatric patients undergoing minor surgery: A 12-year experience with 1,058 patients. *Pediatr Surg Int* 2015; 31: 173-80.
10. Babl FE, Oakley E, Puspitadewi A, Sharwood LN. Limited analgesic efficacy of nitrous oxide for painful procedures in children. *Emerg Med J* 2008; 25: 717-21.
11. Tsze DS, Mallory MD, Cravero JP. Practice patterns and adverse events of nitrous oxide sedation and analgesia: A report from the pediatric sedation research consortium. *J Pediatr* 2016; 169: 260-5.e2.
12. Zier JL, Liu MX. Safety of high-concentration nitrous oxide by nasal mask for pediatric procedural sedation experience with 7802 cases. *Pediatr Emerg Care* 2011; 27: 1107-12.
13. Babl FE, Oakley E, Seaman C, Barnett P, Sharwood LN. High-concentration nitrous oxide for procedural sedation in children: Adverse events and depth of sedation. *Pediatrics* 2008; 121: E528-32.
14. Onody P, Gil P, Hennequin M. Safety of inhalation of a 50% nitrous oxide/oxygen premix - a prospective survey of 35 828 administrations. *Drug Saf* 2006; 29: 633-40.
15. Annequin D, Carbajal R, Chauvin P, Gall O, Tourniaire B, Murat I. Fixed 50% nitrous oxide oxygen mixture for painful procedures: A french survey. *Pediatrics* 2000; 105: e47.
16. Zier JL, Drake GJ, McCormick PC, Clinch KM, Cornfield DN. Case-series of nurse-administered nitrous oxide for urinary catheterization in children. *Anesth Analg* 2007; 104: 876-9.

17. Farrell MK, Drake GJ, Rucker D, Finkelstein M, Zier JL. Creation of a registered nurse-administered nitrous oxide sedation program for radiology and beyond. *Pediatr Nurs* 2008; 34: 29-35.
18. Hennequin M, Manière M-C, Albecker-Grappe S, Faulks D, Berthet A, Tardieu C, et al. A prospective multicentric trial for effectiveness and tolerance of a N₂O/O₂ premix used as a sedative drug. *J Clin Psychopharmacol* 2004; 24: 552-4.
19. Gall O, Annequin D, Benoit G, Van Glabeke E, Vrancea F, Murat I. Adverse events of premixed nitrous oxide and oxygen for procedural sedation in children. *Lancet* 2001; 358: 1514-5.
20. Fink BR. Diffusion anoxia. *Anesthesiology* 1955; 16: 511-9.
21. Duncan GH, Moore P. Nitrous-oxide and the dental patient - a review of adverse reactions. *J Am Dent Assoc* 1984; 108: 213-9.
22. Malamed SF. Sedation: A guide to patient management. 5th ed. Edited by Clark MS, Orr II DL, Reed KL, Soler JG. Maryland heights, Mosby. 2009, pp 235,77.
23. Dunn-Russell T, Adair SM, Sams DR, Russell CM, Barenic JT. Oxygen saturation and diffusion hypoxia in children following nitrous oxide sedation. *Pediatr Dent* 1993; 15: 88-92.
24. Zier JL, Tarrago R, Liu MX. Level of sedation with nitrous oxide for pediatric medical procedures. *Anesth Analg* 2010; 110: 1399-405.
25. Burnweit C, Diana-Zerpa JA, Nahmad MH, Lankau CA, Weinberger M, Malvezzi L, et al. Nitrous oxide analgesia for minor pediatric surgical procedures: An effective alternative to conscious sedation? *J Pediatr Surg* 2004; 39: 495-9.
26. Babl FE, Grindlay J, Barrett MJ. Laryngospasm with apparent aspiration during sedation with nitrous oxide. *Ann Emerg Med* 2015; 66: 475-8.
27. Hornbein TF, Winter P, Smith G, Wetstone D, Smith K. The minimum alveolar concentration of nitrous oxide in man. *Anesth Analg* 1982; 61: 553-6.
28. Zier JL, Doescher JS. Seizures temporally associated with nitrous oxide administration for pediatric procedural sedation. *J Child Neurol* 2010; 25: 1517-20.
29. Amess JA, Burman JF, Rees GM, Nancekievill DG, Mollin DL. Megaloblastic haemopoiesis in patients receiving nitrous oxide. *Lancet* 1978; 2: 339-42.
30. Reynolds E. Vitamin b12, folic acid, and the nervous system. *Lancet Neurol* 2006; 5: 949-60.
31. Stabler SP. Vitamin b12 deficiency. *N Engl J Med* 2013; 368: 149-60.
32. Ilniczky S, Jelencsik I, Kenez J, Szirmai I. MRI findings in subacute combined degeneration of the spinal cord caused by nitrous oxide anaesthesia - two cases. *Eur J Neurol* 2002; 9: 101-4.
33. Remacha AF, Souto JC, Pinana JL, Sarda MP, Queralto JM, Marti-Fabregas J, et al. Vitamin b12 deficiency, hyperhomocysteinemia and thrombosis: A case and control study. *Int J Hematol* 2011; 93: 458-64.
34. Limal N, Scheuermaier K, Tazi Z, Sene D, Piette JC, Cacoub P. Hyperhomocysteinemia, thrombosis and pernicious anaemia. *Thromb Haemost* 2006; 96: 233-5.
35. Dror DK, Allen LH. Effect of vitamin b12 deficiency on neurodevelopment in infants: Current knowledge and possible mechanisms. *Nutr Rev* 2008; 66: 250-5.
36. Honzik T, Adamovicova M, Smolka V, Magner M, Hrubá E, Zeman J. Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin b12 deficiency—what have we learned? *Eur J Paediatr Neurol* 2010; 14: 488-95.
37. McNeely JK, Buczulinski B, Rosner DR. Severe neurological impairment in an infant after nitrous oxide anesthesia. *Anesthesiology* 2000; 93: 1549-50.
38. de Jager J, Kooy A, Leher P, Wulfele MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin b-12 deficiency: Randomised placebo controlled trial. *BMJ* 2010; 340.
39. Gasche C, Berstad A, Befrits R, Beglinger C, Dignass A, Erichsen K, et al. Guidelines on the diagnosis and management of iron deficiency and anemia in inflammatory bowel diseases. *Inflamm Bowel Dis* 2007; 13: 1545-53.
40. Selzer RR, Rosenblatt DS, Laxova R, Hogan K. Adverse effect of nitrous oxide in a child with 5, 10-methylenetetrahydrofolate reductase deficiency. *N Engl J*

- Med 2003; 349: 45-50.
41. Goilmsen J. Agranulocytosis and thrombocytopenia in a case of tetanus treated with curare and chlorpromazine. *Dan Med Bull* 1955; 2: 87-9.
 42. Lassen H, Henriksen E, Neukirch F, Kristensen H. Treatment of tetanus: Severe bone-marrow depression after prolonged nitrous-oxide anesthesia. *Lancet* 1956; 267: 527-30.
 43. Koblin DD, Biebuyck JF. Is nitrous-oxide a dangerous anesthetic for vitamin-b12-deficient subjects. *JAMA* 1986; 256: 716.
 44. Hadzic A, Glab K, Sanborn KV, Thys DM. Severe neurologic deficit after nitrous-oxide anesthesia. *Anesthesiology* 1995; 83: 863-6.
 45. Rosener M, Dichgans J. Severe combined degeneration of the spinal cord after nitrous oxide anaesthesia in a vegetarian. *J Neurol Neurosurg Psychiatry* 1996; 60: 354.
 46. Lacassie HJ, Nazar C, Yonish B, Sandoval P, Muir HA, Mellado P. Reversible nitrous oxide myelopathy and a polymorphism in the gene encoding 5,10-methylenetetrahydrofolate reductase. *Br J Anaesth* 2006; 96: 222-5.
 47. Singer MA, Lazaridis C, Nations SP, Wolfe GI. Reversible nitrous oxide-induced myeloneuropathy with pernicious anemia: Case report and literature review. *Muscle Nerve* 2008; 37: 125-9.
 48. Renard D, Dutray A, Remy A, Castelnovo G, Labauge P. Subacute combined degeneration of the spinal cord caused by nitrous oxide anaesthesia. *Neurol Sci* 2009; 30: 75-6.
 49. Layzer RB, Fishman RA, Schafer JA. Neuropathy following abuse of nitrous oxide. *Neurology* 1978; 28: 504-6.
 50. Huang MY, Tsai W, Chang WH. Nitrous oxide-induced polyneuropathy in a teenager. *Emerg Med J* 2009; 26: 186.
 51. Richardson PG. Peripheral neuropathy following nitrous oxide abuse. *Emerg Med Australas* 2010; 22: 88-90.
 52. Hu MH, Huang GS, Wu CT, Hung PC. Nitrous oxide myelopathy in a pediatric patient. *Pediatr Emerg Care* 2014; 30: 266-7.
 53. Krajewski W, Kucharska M, Pilacik B, Fobker M, Stetkiewicz J, Nofer JR, et al. Impaired vitamin b12 metabolic status in healthcare workers occupationally exposed to nitrous oxide. *Br J Anaesth* 2007; 99: 812-8.
 54. Pichardo D, Luginbuehl IA, Shakur Y, Wales PW, El-Soheby A, O'Connor DL. Effect of nitrous oxide exposure during surgery on the homocysteine concentrations of children. *Anesthesiology* 2012; 117: 15-21.
 55. Nagele P, Tallchief D, Blood J, Sharma A, Kharasch ED. Nitrous oxide anesthesia and plasma homocysteine in children. *Clin Pharmacol Ther* 2012; 91: S102.
 56. Nagele P, Tallchief D, Blood J, Sharma A, Kharasch ED. Nitrous oxide anesthesia and plasma homocysteine in adolescents. *Anesth Analg* 2011; 113: 843-8.
 57. Frasca V, Riazzi BS, Matthews RG. In vitro inactivation of methionine synthase by nitrous oxide. *J Biol Chem* 1986; 261: 15823-6.
 58. Nagele P, Zeugswetter B, Wiener C, Burger H, Hüpfel M, Mittlböck M, et al. Influence of methylenetetrahydrofolate reductase gene polymorphisms on homocysteine concentrations after nitrous oxide anesthesia. *Anesthesiology* 2008; 109: 36-43.
 59. Hakimoglu S, Hanci V, Hakimoglu Y, Cicek S, Yurtlu S, Okyay R, et al. The effects of nitrous oxide on vitamin b12 and homocysteine levels in methyltetrahydrofolate reductase gene mutation. *Bratisl Lek Listy* 2013; 114: 317-22.
 60. Amos RJ, Amess JA, Hinds CJ, Mollin DL. Incidence and pathogenesis of acute megaloblastic bone-marrow change in patients receiving intensive care. *Lancet* 1982; 2: 835-8.
 61. Stabler SP, Allen RH. Vitamin b12 deficiency as a worldwide problem. *Annu Rev Nutr* 2004; 24: 299-326.