

# Does Nitrous Oxide Affect the Corneal Endothelium? A Prospective Randomized Study in Pediatric Patients\*

## Nitröz Oksit Korneal Endoteli Etkiler mi? Pedyatrik Hastalarda Prospektif Randomize Bir Çalışma

### Abstract

**Aim:** In this study, we aimed to evaluate the mean changes in the corneal endothelial cells and pachymetry values of patients who underwent general anesthesia with sevoflurane with N2O or O2.

**Materials and Methods:** Forty eyes of 40 patients who had extraocular surgery under general anesthesia were included in this prospective study. The patients were divided into two groups as the O2 and N2O groups, for which %50 O2 (total flow rate; 2 L/min in O2-air mixture) and %50 O2 (total flow rate; 2 L/min in O2-N2O mixture) were used, respectively. The duration of the surgery was recorded. The CD (cell density), CV (cell variation), Hex (hexagonality rate) and pachymetric specular microscopy results were evaluated before surgery, and at the first and fourth weeks after surgery.

**Results:** The surgical duration and demographic data of the groups were similar, and there was no statistically significant difference between the two groups. The value of CV was  $26 \pm 2$  cell/mm<sup>2</sup> for the O2 group and 30 cell/mm<sup>2</sup> for the N2O group at the first week. There was a statistically significant increase in the N2O group values, compared to the preoperative values ( $p=0.008$ ). The Hex value was  $52 \pm 10\%$  for the O2 group and  $62 \pm 11\%$  for the N2O group at the first postoperative week, and there was a statistically significant increase in the N2O group values in comparison to the preoperative values ( $p=0.007$ ). There was no significant difference between the two groups in terms of CD, CV, Hex, and pachymetry at other times.

**Discussion and Conclusion:** Statistically significant change was observed at the first postoperative week in the corneal variation and hexagonality rate values of the pediatric patients who underwent general anesthesia with nitrous oxide. More studies need to be done for further assessments.

**Keywords:** nitrous oxide; corneal endothelium; child; general anesthesia

### Öz

**Amaç:** Bu çalışmada sevofluran ve N2O veya O2 karışımıyla genel anestezi uygulanan hastaların korneal endotel hücre ve pakimetri değerlerindeki ortalama değişiklikleri değerlendirmek amaçlanmıştır.

**Gereç ve Yöntemler:** Bu prospektif çalışmaya genel anestezi altında ekstraoküler cerrahi uygulanan 40 hastanın 40 gözü dahil edildi. Hastalar N2O ve O2 grubu olarak iki gruba ayrıldı. Her bir grupta %50 O2 (toplam debi; O2 -hava karışımı içinde 2 L/dk) ve %50 O2 (toplam debi; O2-N2O karışımı içinde 2 L/dk) kullanıldı. Ameliyat süresi kaydedildi. Cerrahi öncesinde ve de ameliyattan 1 hafta ve 4 hafta sonra hücre yoğunluğu (CD—cell density), hücre değişimi (CV—cell variation), heksagonalite oranı (Hex) ve pakimetrik speküler mikroskopi sonuçları değerlendirildi.

**Bulgular:** Grupların ameliyat süre ve demografik verileri benzerdi ve gruplar arasında istatistiksel olarak anlamlı fark yoktu. CV değeri birinci haftada O2 grubunda  $26 \pm 2$  hücre/mm<sup>2</sup>, N2O grubunda ise 30 hücre/mm<sup>2</sup> olarak saptandı. N2O grubu verilerinde preoperatif değerlere kıyasla istatistiksel olarak anlamlı bir artış vardı ( $p=0,008$ ). Hex değeri birinci haftada O2 grubunda  $52 \pm 10$ , N2O grubunda ise  $62 \pm 11$  olarak saptandı. Yine N2O grubu verilerinde preoperatif değerlere kıyasla anlamlı artış vardı ( $p=0,007$ ). Diğer zamanlarda CD, CV, Hex, pakimetri verileri açısından gruplar arasında anlamlı bir fark yoktu.

**Tartışma ve Sonuç:** Nitröz oksit karışımı ile genel anestezi uygulanan pediyatrik hastaların korneal varyasyon ve heksagonalite oranı değerlerinde postoperatif birinci haftada anlamlı değişiklik saptanmıştır. Daha ileri değerlendirmeler için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar Sözcükler:** nitröz oksit; kornea endoteli; çocuk; genel anestezi

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## INTRODUCTION

General anesthesia is commonly used in millions of patients worldwide every year, and volatile anesthetic agents are frequently used during general anesthesia. There are many studies on the effects of volatile anesthetics. For example, it was shown that volatile anesthetics could cause endothelial damage by polymorphonuclear neutrophil adhesion (1). Although nitrous oxide ( $N_2O$ ) has been used securely and commonly as an analgesic and sedative or volatile anesthetic, it has also been propounded that  $N_2O$  use during general anesthesia can lead to complications, such as perioperative cardiac arrest and death (2).

Nitrous oxide irreversibly reduces methionine synthase enzyme activity and increases the plasma concentration of homocysteine as a result, and continues to affect for at least one week after surgery (3). Two hours of exposure to  $N_2O$  is associated with a 50% decline in methionine synthase enzyme activity (4). An acute increase in the plasma concentration of homocysteine can cause endothelial dysfunction (5).

The corneal endothelium is composed of single-layer hexagonal cells. The number and formation (density, hexagonality, etc.) of the corneal cells can be reduced by several factors such as aging, trauma, and surgery (6). This causes different cell sizes and appearances (polymegathism), a decrease in the ratio of hexagonal cells (pleomorphism), and an increase in cells with different geometric shapes (7). In this study, corneal cell density (CD), coefficient of variation (CV), hexagonality rate (Hex), and pachymetric specular microscopy results were evaluated before surgery, and at the first and fourth weeks after surgery. CD was used to describe the biological and histological characteristics of the cornea. CD could be high in inflammation areas in the cornea. Hexagonal cell percentage gradually decreases from 75% every year (8).

Nitrous oxide is frequently used with sevoflurane during general anesthesia. Several adverse effects of  $N_2O$  are known, such as megaloblastic anemia, homocysteinemia, thrombosis, neurotoxicity, increased intracranial pressure, cerebral blood flow, expansion of air space and hypoxia, postoperative vomiting, and possible immune suppression (9). However, endothelial effects of  $N_2O$  use on the corneal endothelium have not been investigated thoroughly, and there is insuff-

icient information.  $N_2O$  is also likely to cause toxic effects on the corneal endothelium. The aim of this study was to evaluate the mean changes in the corneal endothelial cells and pachymetry values measured by specular microscopy of pediatric patients who underwent general anesthesia with sevoflurane with  $N_2O$  or  $O_2$ .

## MATERIALS AND METHODS

This study was planned and conducted between January 2017 and July 2017 in accordance with the Helsinki Declaration and the Clinical Research Ethics Committee (reference number 2017-95) of the Inonu University. The study was registered on ClinicalTrials.gov (NCT03006211). Informed consent of the parent(s) of each pediatric patient was obtained. Forty eyes of 40 pediatric patients whose ASA (American Society of Anesthesiologists) scores were I to II and who underwent extraocular surgery under general anesthesia were included in this prospective study. Patients who had uveitis, glaucoma, retinopathy, corneal dystrophy, history of intraocular surgery, history of globe trauma, any systemic disease, and any complication during or after surgery were excluded.

Children aged between 1 and 14 years who underwent general anesthesia were included in this prospective and randomized study. The CD, CV, Hex, and pachymetric specular microscopy results (measured by NSP 9900 noncontact specular microscopy, *Konan Medical Inc., Nishinomiya, Japan*) before surgery, and at the first and fourth weeks after surgery were analyzed. The patients were divided into two groups as the  $O_2$  ( $n=20$ ) and  $N_2O$  ( $n=20$ ) groups. %50  $O_2$  (total flow rate; 2 L/min in  $O_2$ -air mixture) was used in the  $O_2$  group and %50  $O_2$  (total flow rate; 2 L/min in  $O_2$ - $N_2O$  mixture) in the  $N_2O$  group, with sevoflurane use in both groups.

All of the patients were preoperatively evaluated for general anesthesia and stopped consuming solid foods six hours prior to the operation. Midazolam ( $0.5 \text{ mg.kg}^{-1}$ ) administered orally was used for premedication and fifteen minutes later they entered the operating room as part of the routine practice of our clinic. All of the patients were preoxygenated with 100%  $O_2$  6 L/min for three minutes at the beginning of anesthesia. In all of the cases standard anesthesia induc-

**Table 1.** Clinical characteristics of the patients

Characteristics	Group O <sub>2</sub> (n=20)	Group N <sub>2</sub> O (n=20)
Age (yr)	9±3.7	10±2.8
Male sex	11 (55%)	10 (50%)
ASA physical status		
I	17 (85%)	14 (70%)
II	3 (15%)	6 (30%)
Duration of anesthesia (min)	80±15	90±10

Data in n(%) or mean±SD; min: minute; ASA: The American Society of Anesthesiologists

tion (propofol 2 mg.kg<sup>-1</sup> IV, fentanyl 1 µg.kg<sup>-1</sup> IV) was performed and airway control was provided by laryngeal mask airway. Both the O<sub>2</sub> group (%50 O<sub>2</sub> total flow rate; 2 L/min in O<sub>2</sub>-air mixture) and the N<sub>2</sub>O group (%50 O<sub>2</sub> total flow rate; 2 L/min in O<sub>2</sub>-N<sub>2</sub>O mixture) were administered with %1–2 MAC sevoflurane. Parameters for mechanical ventilation were tidal volume; 6–8 ml.kg<sup>-1</sup>, I/E ratio; 1:2. Frequency of respiration was provided with adequate limits to fix EtCO<sub>2</sub> at 35–45 mmHg. Anesthetic agents was stopped and the patients were ventilated by 100% O<sub>2</sub> and extubated when spontaneous ventilation was sufficient at the end of the surgery. The duration of anesthesia was determined as the interval from the induction of anesthesia to the tracheal extubation. The corneal measurements were performed before the surgery, and at the first and fourth postoperative weeks.

### Statistical Analysis

For statistical analysis, Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS, Inc., Chicago, IL) software was used. The Mann–Whitney U and Friedman tests were used to compare the preoperative and postoperative values. The data were denoted as mean±standard deviation (SD). *p* values less than 0.05 were accepted as statistically significant.

## RESULTS

The surgical duration and demographic data of the groups were similar and there was no significant difference between the two groups. The clinical characteristics of the groups are shown in Table 1. The duration of anesthesia for the O<sub>2</sub> group was 80±15 min and 90±10 min for the N<sub>2</sub>O group. The measured changes

in the corneal endothelial specular microscopy values are shown in Table 2. The CV value for the O<sub>2</sub> and N<sub>2</sub>O groups at the first week was 26±2 cell/mm<sup>2</sup> and 30 cell/mm<sup>2</sup>, respectively, and there was a statistically significant increase in the N<sub>2</sub>O group values in comparison to the preoperative values (*p*=0.008). The Hex value was 52±10% for the O<sub>2</sub> group and 62±11% for the N<sub>2</sub>O group at the first postoperative week, and there was again a statistically significant increase in the N<sub>2</sub>O group values in comparison to the preoperative values (*p*=0.007). There was no significant difference between the two groups in terms of CD, CV, Hex, and pachymetry at other times.

## DISCUSSION AND CONCLUSION

The most commonly used agents in general anesthesia are N<sub>2</sub>O, O<sub>2</sub> and sevoflurane. N<sub>2</sub>O is one of the oldest anesthetics and a widely used gas in humans. N<sub>2</sub>O has been used extensively in anesthesia practices for many years, and many studies have been conducted concerning its useful and harmful effects. N<sub>2</sub>O is usually preferred in combination with volatile anesthetic agents for its low potency and taking advantage of seconder gas effect. Our study assessed the effects of N<sub>2</sub>O on the corneal endothelium. The duration of exposure to N<sub>2</sub>O was standardized for each group as approximately 90 minutes. It had been considered that changes in the duration of exposure could affect the results.

It has been shown that some reasons such as trauma, toxicity, surgery, or extra damage affect the corneal endothelium (10). In this study, it was found that N<sub>2</sub>O affected only coefficient of variation and hexagonality rate of the corneal endothelium in the first postoperative week values (Table 2). There was no change in the values at other times because of short-term exposure to N<sub>2</sub>O or the low number of the cases. Pachymetry was used as a marker of acute changes in the corneal endothelium. Besides, the cases were followed up until the fourth postoperative week to investigate the long-term effects of N<sub>2</sub>O on the corneal endothelium.

Several factors such as being female, age, and obesity were defined as factors affecting the toxicity of anesthetic gases for organs (10). The patients in this study were younger and had normal body mass index.

**Table 2.** Changes in the corneal endothelial specular microscopy values

Variables		Preoperative values (mean±SD)	First postoperative week values (mean±SD)	First postoperative month values (mean±SD)	<i>p</i>
CD (cell/mm <sup>2</sup> )	Group O <sub>2</sub>	3063±216	3079±196	3094±226	0.835
	Group N <sub>2</sub> O	3078±231	3145±164	3087±171	
CV	Group O <sub>2</sub>	26±0.4	<b>26±0.3</b>	27±0.3	<b>0.008</b>
	Group N <sub>2</sub> O	25±0.3	<b>30±0.2</b>	28±0.5	
Hex (%)	Group O <sub>2</sub>	51.6±10	<b>52.5±10</b>	52.2±9	<b>0.007</b>
	Group N <sub>2</sub> O	51.5±10	<b>62.5±11</b>	57.1±9	
Pachymetry (µm)	Group O <sub>2</sub>	566±29	569±31	560±38	0.466
	Group N <sub>2</sub> O	574±38	574±36	573±37	

CD: cell density; CV: coefficient of variation; Hex: hexagonality rate; O<sub>2</sub>: oxygen; N<sub>2</sub>O: nitrous oxide

Some studies indicated that volatile anesthetics such as isoflurane or xenon induced preconditioning by decreasing protein kinase C and some protein varieties (11). Polat et al. stated that general anesthesia with sevoflurane did not have a toxic effect on the corneal endothelium and did not change the cell number and cell morphology in the corneal endothelium. N<sub>2</sub>O was not used during general anesthesia (12).

The corneal endothelial cells are not regenerative. The CV and Hex ratios (pleomorphism and polymyatism) in the endothelium are remodeling indicators, and the remodeling of the corneal endothelium after damage can last for up to 3 months (7,13). This may cause a limitation; however, in this study it was considered that N<sub>2</sub>O would not be effective on the corneal endothelium at these doses for up to 3 months. Besides, in a similar study, the duration was limited to 1 month (14). Following studies can be planned with high doses and long-term exposure to N<sub>2</sub>O for up to 3 months, and long-term effects of N<sub>2</sub>O can be investigated.

The effect of N<sub>2</sub>O on vitamin B<sub>12</sub> can cause to endothelial dysfunction. Myles et al. suggested a relationship between plasma homocysteine concentration and endothelial dysfunction after using N<sub>2</sub>O-based anesthesia, and that endothelial dysfunction due to N<sub>2</sub>O use could be a risk factor for postoperative cardiovascular morbidity (15). In their study, the median duration of exposure to N<sub>2</sub>O was greater than 4 hours. N<sub>2</sub>O exposure effects increased postoperative homocysteine levels significantly. Increased plasma homocysteine levels can go on for 1 week after surgery (3). In addition, exposure to N<sub>2</sub>O for more than 2 hours reduce

methionine synthase activity and high plasma homocysteine values at the rate of 50% (4). It was reported in Myles' study that the duration of N<sub>2</sub>O exposure was significantly associated with the grade of endothelial dysfunction (15). They found that endothelial dysfunction was related to increased homocysteine levels and the total reduction of L-arginine and L-citrulline levels after surgery. The inactivation of methionine synthase is associated with the dose of N<sub>2</sub>O, therefore, the dose and the duration of N<sub>2</sub>O exposure should be evaluated for endothelial dysfunction. It has been shown that the rate of enzyme inactivation is over with increased inspired concentration of N<sub>2</sub>O (2). Badner et al. reported that N<sub>2</sub>O exposure was associated with cardiac ischemic events and increased homocysteine levels in ninety patients during general anesthesia (16). In another study, it was shown that risk of myocardial infarction increased after using N<sub>2</sub>O in a clinical study with 2050 surgical patients (2).

Decreases of CD were compensated by way of cell spreading that resulted in increased cellular pleomorphism and a decrease in the percentage of hexagonal cells (6). In some studies it was shown that CD, Hex, and pachymetry decrease with aging and origins (17). Duman et al. reported that mean CV in cell size was 34±7 (range 263–522) and the mean percentage of hexagonal cells was 46±8% (range 25–76%) in a population aged between 6 and 85 years (18). In Elbaz's study, the mean endothelial cell density under the age of 5 was 3746±370 (range 3145–5013) cells/mm<sup>2</sup>. Decline in CD is rapid in the first 2 years of life and this decrease is correlated with age. CD decreases to adult sizes when the cornea reaches its adult form (19). Gao

et al. noticed that central corneal thickness increased in diabetics while CD decreased ( $p < 0.05$ ) (18). Sati et al. suggested that endothelial CD values decreased in patients with chronic renal failure because of raised blood urea level (20). Similarly, it was shown that raised plasma urea levels affected the corneal endothelium and endothelial parameters (4,20). In addition, it was reported that corneal thickness (pachymetry) values increased in dialyzed patients who had chronic renal failure. In this study, there was a significant change only at the first postoperative week in the corneal variation and hexagonality rate values of the pediatric patients who underwent general anesthesia with  $N_2O$ . There was no significant difference between the groups in terms of CD, CV, Hex, pachymetry at other times.

In conclusion, possible effects of  $N_2O$  on the corneal endothelium were noticed in our study. A statistically significant change was observed at the first postoperative week in the corneal variation and hexagonality rate values of the pediatric patients who underwent general anesthesia with  $N_2O$ . More studies investigating the effects of  $N_2O$  on corneal endothelium need to be done for further assessment.

### Limitations

It should be noted that this study has some limitations. First, it did not investigate the plasma homocysteine concentrations in the patients while  $N_2O$  exposure effects increased postoperative homocysteine levels significantly. The correlation between plasma homocysteine concentration and the duration of exposure to  $N_2O$  was not evaluated. Further studies should investigate the effects of the  $N_2O$  exposure duration on the corneal endothelium. Secondly, volatile anesthetics, propofol and opioids might affect the conclusion of our study because of the chance of improving endothelial function (13).

### Ethical Approval Statement

This study was carried out in accordance with the Clinical Research Ethics Committee (reference number 2017/95) of the Inonu University.

### Funding Disclosure Statement

The study received no external funding.

### Conflict of Interest Statement

The authors declare no conflict of interest.

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