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# Association between combined homozygous *MTHFR* C677T and A1298C polymorphisms and adverse effects in Saudi pediatric patients receiving dental treatment under nitrous oxide sedation

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## ABSTRACT

Nitrous oxide ($N_2O$) is a commonly used safe and effective inhalational anesthetic agent in pediatric dentistry. Here, we aimed to evaluate the association between methylene tetrahydrofolate reductase (*MTHFR*) polymorphisms (C677T and A1298C) and adverse effects in nine Saudi pediatric patients who received dental treatment under $N_2O$ sedation using genetic screening tests. Five of the subjects were male (55.6%) and the mean age was 6.8 (±2.35) years. The mean time before the last meal was 6.1 (±4.4) and the mean duration of receiving $N_2O$ was 33 (±14.86) minutes. Only two subjects developed mild to moderate adverse effects, such as nausea, vomiting, dizziness, fatigue, and sleepiness. Genetic screening revealed that both subjects were homozygous for *MTHFR* C677T and A1298C polymorphisms. This suggests a possible relationship between combined *MTHFR* polymorphisms and the development of mild and moderate adverse effects in pediatric patients receiving dental treatment under $N_2O$ sedation.

**Keywords**: Adverse effects, dental sedation, *MTHFR* polymorphisms, nitrous oxide, pediatric

## 1. INTRODUCTION

Nitrous oxide ($N_2O$) is a basic pharmacological management technique used by dentists to reduce children's anxiety during dental procedures according to the guidelines of American Academy of Pediatric Dentistry (AAPD) (Ashley et al., 2018; Lourenço-Matharu et al., 2012). With appropriate patient selection, it is a safe and effective analgesic and anxiolytic agent allowing patients to remain responsive to stimuli and maintain airway reflexes with little effect on the respiratory system in 90% of patients (Paterson et al., 2003; Foley et al.,

2005; Emmanouil & Quock, 2007; Wilson, 2013; American Academy of Pediatric Dentistry, 2016). The most common adverse effects are nausea and vomiting (Kupietzky et al., 2008) which usually occur due to high $N_2O$ doses, fluctuations in levels, or improper titration (Malamed et al., 2003). Although $N_2O$ administration in young children is considered safe (Galeotti et al., 2016), isolated cases of severe adverse effects following the administration of high doses (50%–70%) have been reported (Babl et al., 2015; Chi, 2018). Therefore, lower doses are recommended in young children (Yee et al., 2019).

Methylene tetrahydrofolate reductase (MTHFR) is a vital enzyme in the folate pathway and two polymorphisms (C677T and A1298C) reduce its enzyme activity by 70% (Födinger et al., 2000). Individuals with these polymorphisms are at a higher risk of developing abnormal hyperhomocysteinemia if they are deficient in folate and/or vitamin B12, with fatal outcomes reported in rare cases following exposure to $N_2O$ (Selzer et al., 2003). A recent study reported that 91.29% of Saudi children receiving dental treatment at King Abdulaziz University Hospital had the *MTHFR* A1298C genotype (Bagher et al., 2021).

The aim of this case series was to evaluate the association between *MTHFR* C677T and A1298C polymorphisms and adverse effects in pediatric patients receiving dental treatment under nitrous oxide sedation.

## 2. CASE REPORTS

The study was conducted in the Pediatric Dentistry Department at King Abdulaziz University Faculty of Dentistry (KAUFD) in Jeddah, Saudi Arabia, between November 2019 and January 2020. The study protocol was approved by the Human Research Ethics Committee of the School of Dentistry at King Abdulaziz University (024-03-20).

The inclusion criteria constituted healthy 4 to 12-year-old children requiring dental treatment under $N_2O$. The study aim was introduced to the parents/legal guardians of all patients who met the inclusion criteria during the study period. Those who agreed to participate were asked to sign an Arabic informed consent form before participation. Subjects with a history of dental treatment under $N_2O$ sedation, diagnosed with folic acid or vitamin B12 deficiency, or taking any medications were not considered in this study.

All treatments were provided by a single trained pediatric dentist, and all subjects underwent genetic testing by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to detect *MTHFR* C677T and A1298C polymorphisms using the previously described protocol (Bagher et al., 2021). The investigator performing the genetic test was blinded to the reported adverse effects of the subjects. The AAPD guidelines for dental procedures were utilized for the monitoring and management of pediatric patients during and after sedation. All the subjects were initially administered 100% oxygen ($O_2$) for 5 minutes, and the $N_2O$ concentration was increased incrementally until the desired level of sedation was achieved. At the end of the dental procedure, 100% $O_2$ was administered for 5 minutes to flush out the $N_2O$ from the system and prevent diffusion hypoxia. In addition to $N_2O$ supplementation, the subject was administered 20% benzocaine topical anesthesia gel with a cotton applicator (Sky-Caine Gel, Skydent Inc., NY, USA) for one minute, then, 0.8 mL (case I) or 0.6 mL (case II) 2% mepivacaine with 1/100,000 epinephrine (Scandicaine 2% special, Septodont, UK) using a 27G short needle (Septoject XL, Septodont, UK) before the start of the operative procedure. During treatment, the subjects' level of consciousness, responsiveness to verbal commands and physical stimulation, skin color, and breathing rhythm/rate were constantly observed by the treating dentist and an assistant and the results were recorded every 15 minutes.

Pulse oximetry was utilized and placed on the index finger to monitor the heart rate (HR), respiratory rate (RR), and $O_2$ saturation (Oxy Watch, Choice MMed, Hamburg, Germany), and a proper size nasal hood was selected before treatment initiation. During dental treatment, the behaviors of the subjects were reported based on Frankl's behavior rating scale classification: definitely negative, negative, positive, definitely positive. After the treatment, subjects were monitored for 15 minutes until the pre-$N_2O$ administration level of consciousness, $O_2$ saturation, and HR were regained, and post-$N_2O$ administration instructions were provided to the parent. The parents were contacted by the treating dentist on the same day and the day after treatment to inquire about the development of any side effects after treatment.

## 3. RESULTS

The demographic characteristics of the participants are shown in Table 1. The mean time of the last meal was 6.1 (±4.4), and the mean time of receiving N2O was 33 (±14.86) minutes. Two subjects (22.2%) developed mild-to-moderate adverse effects including nausea, vomiting, dizziness, fatigue, and sleepiness. Genetic screening revealed that the two subjects were homozygous for *MTHFR* C677T and A1298C polymorphisms (Figure 1). The remaining seven subjects who did not develop any side effects were homozygous or heterozygous for either the C677T or A1298C polymorphisms (Table 2).

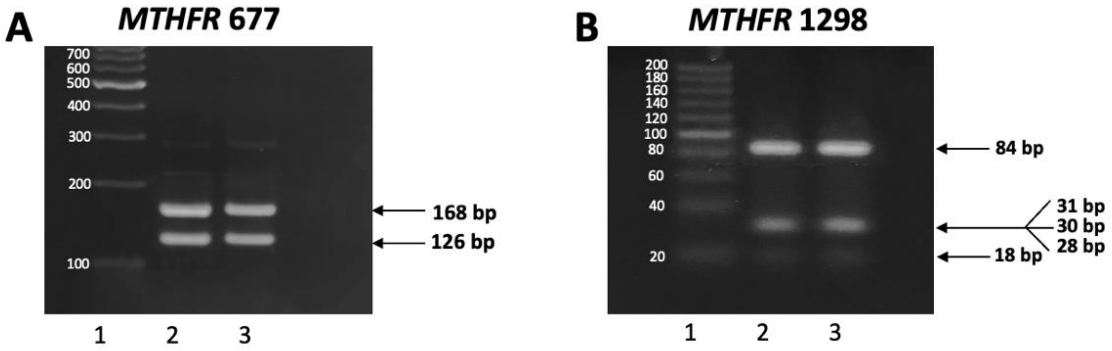


**Figure 1** Polymerase Chain Reaction-Restriction Fragment Length polymorphism (PCR- RFLP) results of *MTHFR* C677T and A1298C polymorphisms. (A) Agarose gel electrophoresis analysis for C677T polymorphism following digestion with *Hinf*. Lane 1: 100 bp DNA marker, Lane 2: sample of case 1, and Lane 3: sample of case 2. Digestion of the homozygous mutant TT genotype yields two bands at 168 and 126 bp. (B) Agarose gel electrophoresis analysis for A1298C polymorphism following digestion with *MboII*. Lane 1: 25 bp DNA marker. Lane 2: sample of case 1, and Lane 3: sample of case 2. Digestion of the homozygous mutant CC genotype yields four bands of 84, 31, 30, and 18 bp. The 84 bp and 30 bp bands were distinctly visible.

**Table 1** Demographic characteristics of the subjects included in the study

| Variables | Mean ±SD |
|---|---|
| Age at treatment day (in years) | 6.8±2.35 |
| | n (%) |
| Gender | |
| Male | 5 (55.6) |
| Female | 4 (44.4) |
| Frankl's behavior scale rating during treatment | |
| Definitely Positive | 4 (44.4) |
| Positive | 3 (33.3) |
| Negative | 1 (11.1) |
| Definitely negative | 1 (11.1) |

**Table 2** Genotype of the *MTHFR* C677T and A1298C polymorphisms of the subjects included in the study. The *MTHFR 677* genotypes were normal homozygous (CC), heterozygous (CT), and mutant homozygous (TT). The *MTHFR 1298* genotypes were normal homozygous (AA), heterozygous (AC), and mutant homozygous (CC).

| Subject # | *MTHFR 677* | *MTHFR 1298* | Side Effects |
|---|---|---|---|
| 1 | TT | CC | Yes |
| 2 | TT | CC | Yes |
| 3 | CC | AC | No |
| 4 | CC | AC | No |
| 5 | CC | CC | No |
| 6 | CT | AA | No |
| 7 | CC | AC | No |
| 8 | CT | AC | No |
| 9 | CC | AC | No |

**Case I**

A five-year-old healthy male patient presented to the dental clinic for restorative treatment the upper right second primary molar. The last meal was 4 h prior to the dental appointment. The baseline HR, RR, and $O_2$ saturation was 104 beats per minute (bpm), 24 breaths per minute, and 98% respectively. After 15 minutes, the HR, RR, and $O_2$ saturation was 100 bpm, 23 breaths per min, and 100% respectively. Similarly, after 30 min, the HR, RR, and $O_2$ saturation was 98 bpm, 24 breaths per minutes, and 100%

respectively. The total treatment time was 41 minutes, and the maximum concentration of $N_2O$ administered was 40% for 15 minutes. Based on Frankl's behavior scale, the child was cooperative during the treatment. Upon contacting the mother, it was revealed that the subject was nauseous, felt dizzy and sleepy, and vomited, with the adverse effects lasting for approximately 24 h.

**Case II**

A four-year-old healthy male patient presented to the dental clinic for pulpotomy and a stainless-steel crown on tooth the upper left first primary molar. The last meal was 3 hours prior to the dental appointment. The baseline HR, RR, and $O_2$ saturation was 74 bpm, 25 breaths per minute, and 99% respectively. After 15 minutes, the HR, RR, and $O_2$ saturation was 82 bpm, 22 breaths per minute, and 100% respectively. After 30 and 45 minutes, the HR, RR, and $O_2$ saturation was 74 bpm, 22 breaths per minute, and 100% respectively. The total treatment time was 45 min, and the maximum concentration of $N_2O$ administered was 30% for 30 min. Based on Frankl's behavior scale, the child was cooperative during the treatment. Upon contacting the father, it was revealed that the subject was sleepy the rest of the day but did exhibit any other adverse effects.

## 4. DISCUSSION

This study examined the possible association between *MTHFR* gene polymorphisms and adverse effects on Saudi pediatric patients receiving dental treatment under $N_2O$ sedation. Two of the nine subjects developed mild to moderate adverse effects following dental treatment under $N_2O$ including nausea, dizziness, fatigue, sleepiness, and vomiting. The genetic tests revealed that both subjects were homozygous for *MTHFR* C677T and A1298C polymorphisms. A limited number of cases with significant side effects in pediatric patients following the administration of $N_2O$ over 80 minutes have been reported. A child exhibited severe neurological side effects resulting in death following anesthesia with $N_2O$ twice within an eight-week period (Selzer et al., 2003). Genetic testing revealed that the child was homozygous for *MTHFR* C677T and A1298C polymorphisms (Selzer et al., 2003). Similarly, Lacassie et al., (2006) reported severe diffuse myelopathy in a patient who received $N_2O$ twice within eight weeks. The neurological symptoms subsided following supplementary therapy with vitamin B12 and folic acid (Selzer et al., 2003). Additional testing showed that the subject was homozygous for the MT*HFR* C677T polymorphism (Lacassie et al., 2006). Thus, it was concluded that MT*HFR* C677T and A1298C polymorphisms may cause hyperhomocysteinemia, which may cause severe side effects following $N_2O$ administration.

Multiple factors are related to negative adverse events associated with the administration of $N_2O$ in dentistry, including lack of experience of the dentist administering the $N_2O$ (Wilson, 2013), administration for a long duration (Zier et al., 2011), lack of titration, and fluctuation of $N_2O$ levels (Emmanouil & Quock, 2007). Therefore, in this study $N_2O$ was administered incrementally by a trained pediatric dentist following the recommended AAPD guidelines for sedation in pediatric patients during dental treatment to minimize the potential side effects associated with $N_2O$ administration (Paterson et al., 2003; Foley et al., 2005; Emmanouil & Quock, 2007; Wilson, 2013; American Academy of Pediatric Dentistry, 2016).

The AAPD does not recommend routine screening for *MTHFR* polymorphisms prior to $N_2O$ sedation administration in pediatric patients. However, AAPD contraindicates the use of $N_2O$ anesthesia in patients with diagnostic *MTHFR* polymorphism. Therefore, alternative anesthetic agents, such as halogenated, oral benzodiazepines, and intravenous anesthetics are considered safe alternatives for patients with *MTHFR* polymorphism (Orhon et al., 2017).

## 5. CONCLUSION

Although $N_2O$ sedation did not cause significantly adverse events, the cases in this study emphasized the possible relationship between combined *MTHFR* gene polymorphism and the development of mild to moderate adverse effects following dental treatment under $N_2O$ sedation in pediatric patients. Further investigations are recommended to identify patients at greater risk of developing these adverse effects.

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**Conflicts of Interest**

The authors declare that there are no conflicts of interests.

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**Informed Consent**

Witten and oral informed consent were obtained from the legal guardians of all the participants included in the study.

**Author's Contribution**

All the authors contributed equally to the case report.

**Data and materials availability**

All data associated with this study are present in the paper.

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