Physiological and Behavioral Manifestations of Nitrous Oxide in Pediatric Dentistry

by

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Abstract

Background: Nitrous oxide (N_2O) is a common anesthetic drug used for distress management and improving the cooperation of children in dentistry. In clinical settings where it is used for moderate sedation and analgesia, the effect of N_2O is mostly perceived as a single action through subjectively observing a child's behavior during a procedure. This can be misleading since N_2O can also have physiological manifestations such as the impact on vital signs as well. Moreover, since moderate sedation limits children's behavioral and communication abilities, the emotions of children are often not appreciated.

Objectives: This study's primary objective was to evaluate and predict the pediatric dental patients' behaviors and vital signs undergoing N_2O and to investigate whether and how behaviors can be predicted from vital signs and vice versa. The secondary objective was to evaluate the effect of age, sex, treatment duration, pre-existing anxiety, and parenting styles on vital signs and behaviors.

Methods: Patients were consecutively recruited from a private dental clinic for this observational study using a within-subject design. All participants received 40% N_2O/O_2 inhalation gas and underwent non-surgical dental procedures. The dental procedure was divided into five different time points namely T1: before administration of N_2O, T2: after administration of N_2O, T3: dental injection, T4: dental treatment, and T5: discontinuation of N_2O and administration of 100% O_2. The primary outcome measure of the study was vital signs including pulse rate (PR), respiratory rate (RR), and oxygen saturation (SpO_2). The PR and SpO_2 were measured using a digital pulse oximeter while RR was measured by counting the number of deflations of the N_2O reservoir bag. The secondary outcome measure, behavior of children, was scored using the Frankl scale through direct observation by a research assistant. Caregivers/parents completed validated questionnaires, including the short version of the Parenting Style and Dimension Questionnaire (PSDQ-32) and the Spence Children Anxiety Scale (SCAS), which were used to measure the parenting style and anxiety, respectively. The demographic information of participants was also collected via a separate questionnaire. The data were analyzed using repeated-measure ANOVA, Friedman test, multiple linear and ordinal regressions as well as general equation estimation (GEE) model at $\alpha=0.05$.

Results: Eighty children with the age range of 2 to 12 years and a mean age of 7.20±2.20 participated in this study. Our results showed that N_2O significantly decreased PR (78.05±8.90 vs. 75.74±8.60), increased RR (22.68±3.52 vs. 23.88±3.07), and improved definitely-positive behaviors (77.5% vs. 57.5%), but did not change SpO_2 (97.41±1.28 vs. 97.48±1.28) meaningfully. There was no interaction between the gas effect and predictors of the study including age, sex, parenting style, anxiety, and treatment duration. The dental injection significantly increased PR (80.13±9.73 vs. 75.74) and worsened the definitely-positive behaviors (55.0% vs. 77.5%) but did not affect RR and SpO_2. Also, the dental treatment itself did not change behaviors and vital signs significantly. Transitioning from 40% N_2O/O_2 to 100% O_2 after finishing the dental treatment did not change behaviors or vital signs. Child age was inversely associated with PR (B=-1.38, P=0.002) and RR (B=-0.26, P=0.003), and the authoritative parenting style predicted cooperative behaviors (odds ratio [OR]=1.93, P=0.01); other predictors were not significant. The higher PR in T2 predicted the poor behavior in T4 (OR=0.65, P=0.006); otherwise, there was no real-time association between vital signs and behaviors.

Conclusion: The results suggested that using N_2O can be considered safe and clinically acceptable by observing only minimal fluctuations in the measured vital signs. Improved cooperation and less distress were seen after the administration of N_2O. During the dental procedure, dental injection

seemed to lead to the highest fluctuations in vital signs and worst behaviors. The possible effect of N_2O was not dependent on the predictors of the study meaning that N_2O can have an impact on vital signs and behaviors regardless of the participants' demographics, degree of anxiety, parenting style, and treatment duration. Vital signs could not be predicted from behaviors, highlighting that the emotions of children cannot be understood by just observing the behaviors.

Preface

This thesis is an original work of Mohammad Moharrami. The research project of which this thesis is a part received research ethics approval from the University of Alberta Research Ethics Board under the project named, "Physiological and Behavioral Manifestations of Nitrous Oxide in the Pediatric Dentistry Considering Sociodemographic Characteristics and Possible Role of Chronic Tolerance", No. Pro00093184, 16/08/2019.

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1. INTRODUCTION

1.1. Nitrous Oxide

1.1.1. Brief History

Nitrous oxide (N_2O) is a neutral, fragrance-free gas that was first discovered in 1793. The term 'laughing gas' was coined in 1799 by Sir Humphrey Davy who used N_2O to treat patients in the Pneumatic Institute. The term 'laughing gas' was used because of the euphoric effects of N_2O when inhaled, a characteristic that sometimes leads to its recreational use and misuse. Sir Davy noted the analgesic effects of N_2O and suggested its administration in alleviating pain during minor surgeries. However, it was not until the early 1840s that N_2O found its place in the field of healthcare and was recognized as an anesthetic drug in dentistry and medicine. Thereafter, N_2O was used widely for dental anesthesia, but eventually, its application decreased in a period following reports of hypoxia and even death when it had been used in the highest concentrations. However, during the past 150 years, methods of administrating N_2O were changed dramatically, and it began to be used widely for its anesthetic effect in dental and medical fields. The induction of anesthesia through N_2O is considered a great achievement in dentistry, comparable to the discovery of local anesthesia and the fluoridation of water (1,2). N_2O use increased in the 1970s and 1980s, then decreased for a short period of time due to environmental concerns, but started to increase again by the beginning of the 21st century (3).

By the 1980s, parents had gained awareness of the need to protect their children from unnecessary pain and distress and requested alternative and additional treatment methods to the nonpharmacologic techniques (e.g. tell-show-do followed by positive reinforcement) that were typically employed at the time. At this time, N_2O and general anesthesia (GA) were ranked eighth and ninth for preferred methods of pain and distress management in dentistry (4). However, things changed quickly changed over time such that, by 1991, N_2O was ranked second after the tell-show-do technique among eight different methods used for pain and distress management (5). The same trend continued such that in 2005 when another survey showed that among the same eight behavioral management modalities, N_2O remained second after tell-show-do, but GA ratings increased and fell in third place (5,6). Currently, N_2O is used commonly in many general and pediatric dental practices (7).

1.1.2. Necessity and Conditions for Using Nitrous Oxide

There is a high demand for sedation in pediatric dentistry. In some cases, it may take up to 12 months of waiting time for a single tooth extraction to be scheduled under GA (8). Therefore, a behavioral and pharmacological hierarchy is needed to meet and manage this high demand (8). Equipping dentists with the necessary knowledge and skills to implement alternative options can greatly help reduce dissatisfaction resulting from long waiting times in hospital settings. While GA is the only option for some patients, the ability of dentists to provide moderate sedation for a routine dental practice can help to improve the patient experience by reducing pain, anxiety, and waiting time (9). There are two main methods for administrating sedative agents in pediatric dentistry, inhalation and oral routes. Induction of moderate sedation using inhalation gases such as N_2O has become more common in recent years due to limitations of the oral route administration. Examples of oral drugs used in dentistry are chloral hydrate and midazolam. Unfortunately, the effects of oral drugs are less predictable compared to inhalation gases. Unlike inhalation gases, when using oral drugs, sedation dose cannot be controlled during the treatment. Also, extra specialized training is needed for oral health practitioners to administrate the oral drugs (8).

N_2O is suggested as being useful for mildly to moderately anxious pediatric patients who can comprehend and act on simple instructions. Other patients outside this spectrum may be managed using nitrous oxide as well but the success is less predictable. N_2O is particularly recommended for patients having their first visits who worry about pain and anticipate that the treatment session to be unpleasant. As a result of using N_2O, direct patient care time increases, and clinicians can manage time more efficiently. Also, N_2O is beneficial for children who cannot handle the difficulty of multiple dental visits (7). The typically recommended concentration of N_2O is 40% for dental practices, but different variations are considered acceptable as well due to variations in sensitivity, clinician expectations of the intensity of sedation, procedural time, and mask fit (10).

1.1.3. Biological Effects and Mechanisms of Actions

It is only in recent years that mechanisms of actions of N_2O including analgesic, anxiolytic, and anesthetic effects have been described (11). There is evidence that the anxiolytic and anesthetic effects of N_2O are independent of, and different from, its analgesic action (2). N_2O has a depressive effect on the central nervous system (CNS) enough to produce mild to moderate analgesia (7). The chain of actions starts with the release of endogenous opioid peptides which in turn activates opioid receptors and descending gamma-Aminobutyric acid (GABA) and noradrenergic pathways that control nociceptive mechanism at the spinal level. The anxiolytic effect of N_2O is processed through the activation of GABA receptors at the benzodiazepine binding sites which consequently leads to activation of three enzymes, namely nitric oxide (NO), soluble guanylyl cyclase, and protein kinase G (PKG). The anesthetic effect of N_2O originates from inhibition of N-methyl-D-aspartate (NMDA) glutamate receptors which have an excitatory influence on the nervous system (11).

Although N_2O is the most widely used anesthetic drug in the medical field, it has limited potency. The minimum alveolar concentration of N_2O is 104% in humans, which means that if it is used alone, a high volume and hyperbaric conditions are needed in order for it to have anesthetic effects in 50% of patients (12). Therefore, N_2O is mostly used for its *second-gas effect* by practitioners to reduce the minimum alveolar concentration of a second inhalation anesthetic drug and also to induct the analgesic effect (13). The subanesthetic concentrations of N_2O are effective in providing analgesia and anxiolysis but not unconsciousness (14). Although N_2O increases the stimulus detection threshold and improves pain tolerance, it cannot eradicate pain when it is used in concentrations below 50%. One noteworthy point is that the analgesic effect of N_2O is significantly impacted by positive verbal reinforcement which can increase pain tolerance. Some authors even suggest that if the benefits of using N_2O are not explained in the way that patients can form particular expectations, pain threshold and tolerance may not be changed and in some cases even decrease (15,16). Therefore, at lower doses of N_2O, it seems analgesic effects and, probably to some extent, the sedative and anxiolytic properties are highly related to patient preparation by providing detailed information about the benefits of the anesthetic drug (3).

1.1.4. Respiratory Effects

When the delivery of N_2O is stopped, the alveolar-arterial gradient works in favor of the movement of N_2O from the blood into the alveolus. As a result, oxygen dilutes out leading to diffusion hypoxia. Hypoxia is considered as one of the important respiratory effects of N_2O (17,18). To cope with this problem, supplemental oxygen is delivered to counterbalance the clinical impact of diffusion hypoxia. Most volatile anesthetic drugs including N_2O can affect respiratory drive, and there is a high chance that they decrease minute ventilation by affecting the central control of respiration. That being said, when N_2O is used without combining it with other anesthetic drugs, it has partial effects on respiratory and cardiovascular functions (19,20). N_2O has a limited effect on respiratory depression compared to other volatile anesthetic drugs (21,22). The depressive effect of N_2O on the respiratory system causes a decrease in tidal volume and an increase in the respiratory rate. Since similar to most volatile anesthetic drugs, the increased respiratory rate does not completely neutralize decreased tidal volume, the minute ventilation is decreased eventually.

1.1.5. Cardiovascular Effects

Similar to respiratory function, when evaluating the cardiovascular effect of N_2O, it should be noted that any impact may be altered by the patients' health and the presence of comorbidities. Generally, N_2O causes a partial decrease in blood pressure, heart rate, and systemic vascular resistance due to depression of myocardial contractility (23–25) The reason behind myocardial contractility is attributed to a decrease in the availability of intracellular cytosolic calcium while not changing the sensitivity of the contractility apparatus and its responsiveness to intracellular calcium (19,23). In many cases, the myocardial effect or the systemic vascular resistance is counterbalanced by stimulation of the sympathetic nervous system (26).

1.1.6. Advantages and Benefits

In the medical field, N_2O is administrated routinely in many operating rooms worldwide as part of the intraoperative anesthetic regimen, usually in combination with other anesthetic drugs such as isoflurane, sevoflurane, and desflurane (27). A unique aspect of inhalational anesthetic drugs such as N_2O is their absorption through respiratory tracts. The blood-gas solubility coefficient, which describes the relative concentration of an anesthetic drug between the blood and the alveolar gas when they are in equilibrium, is used to define the onset and duration of action of an anesthetic drug. Compared to other volatile anesthetic drugs such as sevoflurane, halothane, isoflurane, N_2O is considered to be insoluble in blood. For example, the blood-gas coefficient for N_2O is 0.47 while it is 2.5 for halothane. This low solubility in blood and fat gives N_2O two advantages over other anesthetics, rapid onset and reversibility upon discontinuation. The rapid onset and offset are clinically important for procedural sedation and are considered the main reasons for the routine use of N_2O (27,28). Besides that, N_2O has other advantages that make it favorable to be used in anesthetic procedures such as its relatively easy use, inexpensive cost, and administration through the respiratory system which eliminates the need for intravenous access (27).

In dentistry, N_2O is intended for mild and moderate sedation for routine dental procedures in anxious patients (29). Mild and moderate sedation can have an impact on consciousness, cognition, motor coordination, anxiety level, and physiological responses. The main characteristic of mild and moderate sedation is the ability of patients to respond to verbal commands either on their own or when light tactile stimulation is given (30). In pediatric dentistry, N_2O greatly helps to manage moderately anxious children and deliver better treatments. Some unique characteristics of N_2O making it favorable in dental settings are safety, anti-anxiety effect, pain-relief effect, and specifically, rapid onset and reversibility upon discontinuation (8,31,32). N_2O/O_2 inhalation is recognized by the American Academy of Pediatric Dentistry (AAPD) as a safe and useful method in reducing anxiety and pain and enhancing communication between a patient and health care provider (30).

1.1.7. Side Effects and Limitations

Similar to other anesthetic drugs used in procedural sedation, there are some downsides to the use of N_2O as well (27). Practitioners may find the use of N_2O completely safe given its routine use in dental practices with only a few reports of safety concerns and side effects (33,34). However, in many of the previous reports, physiological responses were not monitored, or standard guidelines were not followed completely (35–37). The reported rate for side effects of N_2O is about 0.3%

with vomiting being the most prominent adverse event. Also, cases of laryngospasm were reported following the use of high-dose N_2O. Notably, neurological symptoms were common among patients who were exposed repeatedly to N_2O, and those who had vitamin B_{12} deficiency (38). Furthermore, some contraindications have been named for N_2O such as having chronic obstructive pulmonary diseases (39), drastic emotional problems (40), being in the first trimester of pregnancy (41), and being treated with bleomycin sulfate (42). Besides that, having methylenetetrahydrofolate reductase deficiency is considered another risk factor (43). Further, it is recommended that medical consult should be sought for patients with obstructive pulmonary disease, congestive heart failure, sickle cell disease (44), acute otitis media, recent tympanic membrane graft (45), and acute severe head injury (46).

A recent narrative review of 180 published papers by the European Society of Anesthesiology stated that: "*Many perceived drawbacks of medical N2O administration (e.g. nausea, vomiting, use during laparoscopy, cardiac ischemia, environmental effects) have been exaggerated or misplaced…We recommend that the supply of N2O in hospitals be maintained while encouraging its economic use* (47) ." It should be noted that similar to any other medical drug, the use of N_2O is subject to an individual's health and conditions, for example, extra caution should be taken in vegetarian children who might have a higher risk of vitamin B_{12} deficiency. Overall, the 50% concentration of N_2O mixed with O_2 is suggested to provide the highest benefits and the lowest risks (48).

Similar to many anesthetic drugs, the adverse and other side effects of N_2O are considered to be generally dose dependent. Although not accepted worldwide, some practitioners suggest that the preparation and monitoring of patients according to guidelines developed for deep sedation may not be needed to be followed strictly when the concentration of N_2O is not above 50% (35–

37). One important point when interpreting the results of previous reports is the actual concentration of N_2O used in dental settings. In dental settings, a nasal hood is used to deliver the anesthetic agent so that patients can open their mouth freely during dental treatments. Therefore, the concentration of N_2O leaving the device is not equal to the actual inhaled concentration of N_2O that reaches the alveoli. The reasons behind this incongruity are the leakage as a result of poorly fitting nasal masks, dead space, and mouth breathing (49,50). It is speculated that in dental settings if the concentration is set at 50% to 70%, the actual concentration delivered to alveoli is expected to be 30% to 50% (27).

1.1.8. Combination of Nitrous Oxide with Other Anesthetics

In dentistry, only a few studies have evaluated the effect of N_2O without combining it with other anesthetics (51–53). One of the common anesthetics used in combination with N_2O in previous studies is midazolam. Midazolam is a preoperative sedative agent and belongs to the family of short-acting benzodiazepines. Besides dentistry, midazolam is often used in emergency and oncology departments to provide mild sedation and anxiolysis for pediatric patients. There are some advantages to midazolam such as easy administration, short onset of action, excellent amnesia, and a good safety profile (54). Similar to N_2O, using oral midazolam does not lead to considerable respiratory depression (54,55).

The combination of midazolam and other anesthetic drugs with N_2O is usually safe since N_2O is carried in a physical solution alone without binding to any carrier protein; therefore, the risk of pharmacokinetic interaction is minimized (56). However, a combination of N_2O with other anesthetics can lead to deeper sedation (56,57) which is different from general anesthesia only by some degree of arousal or voluntary movement (35). Further, it may be possible that the additive effects of anesthetic drugs manifest into different physiological and behavioral responses.

Therefore, studies that evaluate the effect of N_2O in combination with other drugs can only provide a general understanding of the effect of N_2O and caution should be taken when interpreting their results.

1.2. Emotion

1.2.1. Definition

Imparting values to events surrounding a living organism is an ability that evolved during the selective processes (58). Emotions can be defined as *psychological and physiological states* that mark the manifestations of personal values. The importance of emotions for human experience is evident from the variety of feelings such as joy, sadness, pleasure, etc. and the fact that what humans perceive and remember is not neutral. Emotion provides a reference point for human relationships and behaviors in terms of defining what is the best or worst motivation for a conscious organism. Emotions also have a strong impact on reasoning and play an important role in the fixation of belief, which has not been totally understood or scientifically investigated. Due to obvious intellectual capacity and self-consciousness, humans benefit and suffer most from emotional experiences (59).

Emotion, different from most psychological states, have certain properties that make it unique and recognizable. For example, emotions are stereotyped, which can be seen and detected in facial expression and autonomic arousal. Emotions are unusually triggered and are not bonded with human's intentions like other psychological states (59). From the neurological point of view as described by Pace-Schott et al., emotions are "*a programmed neural response evolved to serve an adaptive function by mobilizing specific neural activity in both the brain and periphery and by favoring certain behaviors. An emotional response can be evoked by, generate, or be shaped by specific feelings as well as by specific exteroceptive stimuli, cognitions or cognitive processes*

(60).” In conducting research, emotional responses and feelings must be differentiated. Most of the time these terms, emotions and feelings, are used interchangeably which should be avoided in scientific research (61,62). Feelings can be defined as mental representations of physiological responses that follow and describe the emotional provoking conditions or states (59).

1.2.2. Measurement

Based on a consensual model, emotions are physiological and behavioral responses to meaningful stimuli on a personal level (63). Therefore, based on this model, emotions consist of three responses, namely physiological, behavioral, and subjective experience/perception. As a result, there is no gold standard to measure emotions, and all the three mentioned responses are important and cannot be presumed to be interchangeable (63). From the scientific point of view, measuring a person’s emotional state is one of the most challenging issues in affective research (63).

In terms of measuring each component of emotion, perception is probably the most difficult one. It has been shown that current emotional experiences are more valid measurements than self-reports that measure the experience of an individual sometimes in the future after the event takes place (64). The report of emotions after an event/treatment may not be a reliable representation of an emotion as it has been shown that momentary experience and memory do not always agree with each other (65). Even when we intend to measure an experience concurrently with an event, there are some concerns such as the ability of individuals in reporting on their momentary emotional states. In dental settings, measuring the momentary experience is even more challenging since the children who are undergoing N_2O have a nasal hood and as a result, they cannot describe their experience while under treatment. Therefore, measuring the momentary experience of children in a real dental setting is not feasible, and controlled condition that interferes with the real settings is needed. While important, in this study, we did not focus on the perception of children as a part of emotional response since we intended to measure behavioral and physiological responses in a real dental setting. Based on the results, it will be discussed if and how future studies could incorporate the perception component.

Besides the CNS, the autonomic nervous system (ANS) forms part of the physiological response related to emotions. The ANS consists of sympathetic and parasympathetic divisions responsible for modulating peripheral functions (64). Sympathetic and parasympathetic divisions are responsible for activation and relaxation, respectively (66). However, the literature does not support a one to one relationship between a discrete emotion and distinct autonomic responses. Instead, previous studies have shown that relationships exist between dimensions, i.e. valence and arousal, and ANS responses. Perhaps multiple ANS measures will eventually show the autonomic specificity of emotions, but more research is needed to draw that conclusion (63). In this study, we measured three important vital signs namely pulse rate, respiratory rate, and blood oxygen saturation as proxies of the physiological response.

According to Darwin (1965), emotions have been evolved to foster communication; therefore, related behaviors have evolved alongside it (67). The primed tendency toward flight in the case of fear is one example that some theories use to link emotions and behaviors (68). Different behavioral measures including vocal characteristics, facial behavior, and whole-body behavior are used to describe emotions (63). However, it should be noted that several factors such as sex, cultural values, expressiveness, and the presence of an audience, can potentially change the relations between emotional states and behaviors. Therefore, caution should be taken not to interpret the absence of behavioral changes equal to the absence of corresponding emotions, and vice versa (63). In this study, we used the Frankl scale, a validated and widely used tool in dental settings to measure children's behaviors (69). This scale classifies the behaviors of children, based on their cooperation during their dental treatment, into four categories of definitely negative, negative, positive, and definitely positive (69,70).

1.3. Factors Affecting Behaviors and Physiology

When measuring the effect of N_2O on behaviors and vital signs, the possible role of confounding factors should be considered so that the true effect of N_2O can be pointed out. It is well documented in the literature that vital signs including pulse rate and respiratory rate vary significantly by age and sex (71–73). Some studies even showed that age and sex can be risk indicators for fluctuations of vital signs during conscious sedation (74) and also the predictors of behavioral distress during painful procedures (75–77).

Besides demographics, another factor that can predict children’s behavior and cooperation is parenting style. Based on the topology suggested by Baumrind, parenting style can be categorized into authoritarian, authoritative, and permissive (78). In the authoritarian style, parents are strict and make sets of rules for children that should be followed. As a result, children are not usually free to make their own decisions and if they disobey their parents, there will be severe punishments. In the authoritative style, children have more freedom to make their own decisions and learn from their mistakes, and parents just set some boundaries and provide guidance. In the permissive style, few limits and boundaries are defined for children (78). It has been shown that parenting style can mediate children's temperament and anxiety. For example, the authoritative parenting style related to a positive child's behavior but inversely related to a child's anxiety (79). Authoritative parenting also related to less dental caries and more cooperative behaviors specifically during the child's first dental visit (80). Among different types of treatments, it was shown that parenting style can predict children’s behaviors specifically when they are going under restorative dental treatments (81).

Children's degree of anxiety is another factor that may affect behaviors and vital signs. There is some evidence that the anxiety of children, regardless of their age, can have an impact on their behaviors during dental treatment. Therefore, it is important to evaluate and interpret the degree of anxiety for each child and ideally scheduling the treatment plan accordingly (76,77,82). Another factor that was found important in predicting behavior and scheduling treatment plans in pediatric dentistry is treatment duration (83). Children's behaviors usually worsen when treatment duration increases and they tend to cooperate less with their health providers (84). To date, these confounding factors are seldom measured and rarely used to interpret the results regarding the effect of N_2O on vital signs and behaviors of children.

1.4. Objectives

To the best of our knowledge, few studies have evaluated the effect of N_2O/O_2 on vital signs or behaviors without combining it with other sedative agents (51–53). However, even these few studies either did not evaluate both vital signs and behaviors concurrently or did not follow patients' behaviors and vital signs throughout the dental procedure starting prior to administration of N_2O to its discontinuation and administration of O_2. Among these three studies, one evaluated the effect of N_2O on both vital signs and behaviors but only compared the results between N_2O and O_2 groups at baseline. This study also did not investigate the relationship between behaviors and vital signs (51). The other study followed patients throughout the dental procedure, but only reported the results for respiratory rate but not pulse rate or oxygen saturation (52). The study by Bonafé-Monzó recruited adult participants in a non-dental setting (53). Therefore, to address the existing literature gaps, the objectives of this study were to:

1. Evaluate and predict the pediatric dental patients' behaviors and vital signs including pulse rate, respiratory rate, and oxygen saturation level before and after administration of N2O, during dental treatment, and after administration pure O_2.
2. Examine the predictive value of vital signs for behaviors and vice versa.
3. Explore the moderating/confounding effects of age, sex, treatment duration, pre-existing anxiety, and parenting styles on vital signs and behaviors.

2. METHODS and MATERIALS

2.1. Study Design and Ethical Approval

The observational cohort design was employed to conduct this study (85). The study protocol was reviewed by the Health Research Ethics Board-Health Panel of the University of Alberta, and ethical approval (ID: Pro00093184) was obtained on August 16, 2019. The purpose, procedure, and possible risks, discomforts, and benefits of this study were explained to parents/guardians of children. Consent was obtained from all parents/guardians of eligible children before being recruited to study. Assent was obtained from children who were 7 years or older after the dental treatment was finished.

2.2. Setting and Participants

Participants were recruited from the Childrens Dental Clinic, in Edmonton, AB. This pediatric dental clinic has seven operating rooms, four dentists, one hygienist, and 6 assistants. In this clinic, dentists usually see 20 children per day and perform up to 12 procedures with N_2O daily.

The following inclusion criteria were used to recruit children:

a) between 2 to 12 years,
b) who supposed to receive N_2O/O_2 as part of their standard treatment,
c) who were fluent in English,
d) who needed restorative dental treatment such as pulpotomy, amalgam filling, composite filling, and stainless-steel crowns,
e) who, based on the American Society of Anesthesiologists (ASA), were classified as normal healthy patients (ASA I).

Children were excluded if they:

a) had been exposed to N_2O within the last 30 days,

b) had a history of post-traumatic stress disorders or specific phobia related to the dental settings,

c) had a known allergy or previous adverse reactions to N_2O,

d) needed invasive surgical dental procedures such as dental extractions,

e) had waited in the waiting room for more than one hour,

f) had severe or chronic orofacial pain or chronic systemic disease,

g) had dental injections in the past 30 days.

2.3. Recruitment

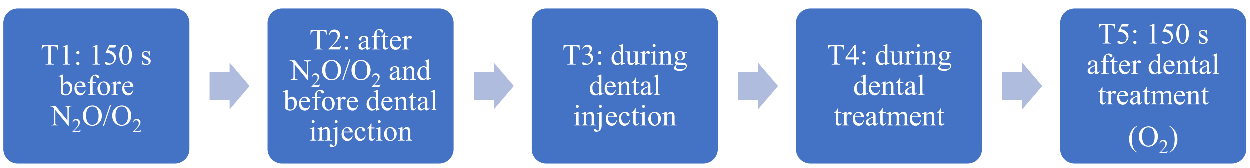
Before starting the dental procedure, the MSc student provided a brief explanation of the purpose and process of the study based on the prepared information sheet to parents/guardians of eligible children. All parents/guardians were provided with a copy of the information sheet and signed the consent form after discussing any questions or concerns they might have had. Also, children 7 years or older provided with an assent form just after the dental session was finished as discussed in the ethics application. Since the assent form included information about the aims and process of the study, and the fact that we were trying to evaluate the patients' behaviors and vital signs, if children knew beforehand about the study, it could have affected the measurements. Therefore, the results would have been different from what one could see during the real clinical conditions.

2.4. Procedure

While in the operatory room, the parents/guardians completed the questionnaires including the short version of Parenting Styles and Dimensions Questionnaire (PSDQ-32), Spence Children's Anxiety Scale, and demographics. The clinical dental procedure took place in 5 different time points (Fig. 1):

- (T1) before inhalation of N_2O/O_2
- (T2) after inhalation of N_2O/O_2
- (T3) during the dental injection
- (T4) during the dental treatment
- (T5) after finishing the procedure and discontinuation of N_2O/O_2 and administration of 100% O_2.

Figure1. **Description of the five time points of study (T1 to T5)**



In T1, the dental assistants sat the child on the dental unit, turned on the TV above the dental unit, and positioned the headphone for children to provide the audio-visual distraction. In T2, the dentist put on the nasal hood for the children to administer the N_2O/O_2 gas; the children were inhaling N_2O/O_2 approximately 5 minutes before starting the next step. In T3, benzocaine 20%, (Master Dent Topical Anesthetic Gel, USA) was applied on dried mucosa for 1 minute, and then local anesthesia, 2% lidocaine and 1:100,000 epinephrine (Cook-Waite Lidocaine, Septodont, USA) was administrated using controlled local anesthetic delivery system (The Wand, Milestone Scientific, USA). In T4, standard dental treatments were delivered based on the previously approved treatment plan. The rubber dam was not used for any of the treatments since the dentists used a new isolation system (Isolite 3, Zyris, USA) in this clinic.

Using a portable nitrous oxide unit (MRX, Porter Instrument Co., USA), the combination of the N_2O/O_2 was administrated at a concentration of 40/60 via nasal hood at the flow of 5 liters/minute in T2, T3, and T4. In T5, after finishing the dental treatment, N_2O/O_2 was discontinued and 100% O_2 was provided. The same dentist who administered the N_2O/O_2 and O_2 performed the dental treatment. The restorative dental treatments were performed by two experienced pediatric dentists who were working together for the past 10 years.

Children's behaviors were scored using Frankl's scale for the total procedure and also at each time points. Vital signs including pulse rate (PR) and oxygen saturation (SpO_2) were measured every 4 seconds during each time point using a digital oximeter attached to the thump or index finger of children. Then, the mean values for each of the five points and the whole procedure were calculated. The respiratory rate (RR) was calculated by counting the number of squeezes of the N_2O reservoir bag as the system is patient driven. The total duration of dental treatment from the beginning of T1 to the end of T5 was calculated in minutes.

2.5. Instruments and Measures

2.5.1. Independent Variables

2.5.1.1. Parenting Styles and Dimensions Questionnaire

Parenting Styles and Dimension Questionnaire (PSDQ) evaluates parenting style through self-reports of parents by measuring the usage of various parental practices and parental reactions to children's behaviors (*Appendix 1*) (86). This questionnaire classifies parenting style into authoritarian, authoritative, and permissive based on the topology suggested by Baumrind (78). PSDQ has been found as one of the few instruments reliable to classify parenting style (87). In this study, we used the 32-item short version of the questionnaire (PSDQ-32) (88) from the original 62-item questionnaire (86), which was developed by using structural equation modeling (SEM) and recruiting 1900 parents of preschool and school-aged children (88). PSDQ-32 is a validated and reliable questionnaire that has been used worldwide in different cultures due to the convenience of application and interpretation. A review paper conducted in 2013 showed that PSDQ was used mostly in the studies in North America (58.49%) followed by Asia (18.87%), Europe (15.09%), Oceania (5.66%), and Africa (1.89%) (89).

The PSDQ questions were developed based on a 5-point Likert scale, and parents can score each statement from 1 (never) to 5 (always). There are 12 questions such as "I scold or criticize when our child's behavior doesn't meet our expectations" for the authoritarian style with scores ranging from 0 to 65. There are 15 questions such as "I give our child reasons why rules should be obeyed," for the authoritative style with scores ranging from 0 to 75. There are 5 questions such as "I spoil my child" for the permissive style with scores ranging from 0 to 25. The highest mean score in each category determines the dominant parenting style. According to Robinson et al., the English version of the PSDQ has acceptable internal consistency and reliability and can be completed by either of parents. The Cronbach's alpha value for the authoritative, authoritarian, and permissive parenting scales is 0.86, 0.82, and 0.64 respectively (88).

2.5.1.2. Spence Children's Anxiety Scale

This widely used validated scale provides two related parent-report questionnaires categorized based on the age of participants, the Spence Children's Anxiety Scale, Parent Report (SCAS-P) which is used for school-age children (*Appendix 2*) (90) and Preschool Anxiety Scale (PAS) which is appropriate for pre-school children (*Appendix 3*) (91). Both of these scales originated from the main scale (SCAS) developed in 1998 (92). SCAS provides an overall score for anxiety and can also be used to categorize children's anxiety symptoms into six subscales derived from the Diagnostic and Statistical Manual of Mental Disorders, 4^{th} edition (DSM-IV) namely generalized anxiety, social anxiety, obsessive-compulsive disorder, physical injury, fears, and separation anxiety. The PAS questionnaire consists of 28 items such as "has difficulty stopping him/herself from worrying" that parents can score based on the Likert scale from 0 (not true at all) to 4 (very often true). The maximum score is 112, and higher the score higher the anxiety symptoms (90). SCAS-P consists of 38 items such as "my child worries about things" that parents can score from 0 (never) to 3 (always). The maximum score is 114, and higher scores correlate with higher anxiety symptoms (91).

2.5.1.3. Demographics and Treatment Duration

The only demographic information used in this study were age and sex of children reported by their parents since these two variables have been shown to affect the results in previous studies. The treatment duration was measured using the pulse oximeter (Fig. 2) from the beginning of the T1 to the end of the T5 and reported in minutes.

Figure 2. **Lookee Pulse Oximeter**

2.5.2. Outcome Measures

2.5.2.1. Vital Signs

PR, SpO_2, and RR were the three vital signs measured in this study. The PR and SpO_2 were measured using an FDA listed pulse oximeter (Lookee Pulse Oximeter). The pulse oximeter was attached to the thumb or index finger of children and was securely connected to the researcher's smartphone, and real-time information was recorded without any interruptions every 4 seconds. The pulse oximeter's range was 30 to 250 bpm for PR and 70% to 100% for SpO_2. The beginning of each time point was noted so that when analyzing the data, the records for each time point could be separated. The RR was measured by counting the number of squeezes of the N_2O reservoir bag every 30 seconds in each time point, multiplied by 2, and then reported per minute. If a time point (for example T4) took longer than 5 minutes, the respiratory rate was measured every 5 minutes and the average of the measurements was reported (93).

2.5.2.2. Behaviors

Behaviors of children were scored using the Frankl scale, a validated and reliable tool for dental settings (*Appendix 4*). The Frankl scale classifies the behaviors of children based on their cooperation during the dental treatment into four ordinal levels of Definitely Positive (F-score: 4), Positive (F-score: 3), Negative (F-score: 2), and Definitely Negative (F-score: 1) (69,70). The MSc student, who did not have any previous information on demographics, parenting style, and degree of anxiety of any participants, scored children's behaviors during the treatment by standing behind the dental unit where the dentist was performing the treatment, so the child undergoing the treatment could not see the researcher. The behaviors were scored for each time point separately. The total behavior for the whole procedure was scored based on the general behavior demonstrated in the whole procedure (70).

2.6. Sample Size Calculation and Data Analysis

Sample size was calculated using GPower v3.1.9 software (94). Based on the information from a previously published study, the mean RR was 22.27 (standard error=0.31) for 40/60% N_2O/O_2 and 23.25 (standard error=0.28) for 100% O_2 (52). Based on this information the effect size (dz) of 0.70 was obtained for the paired t-test. Considering power (1-β) of 0.80 and alpha error of 0.05, the total samples of *19* were adequate for a two-tailed analysis.

Based on the information from another study, the mean PR was 89 (standard error=0.6) for 40/60% N_2O/O_2 and 87 (standard error=0.5) for 100% O_2 (51). Based on this information the effect size (dz) of 0.76 was obtained for the paired t-test. Considering power (1-β) of 0.80 and alpha error of 0.05, the total samples of *16* were adequate for a two-tailed analysis.

For conducting simple linear regression analysis, considering the medium effect size (f^2)=0.15, alpha error=0.05, statistical power (1-β)=0.80, 55 samples were enough. Based on the results of simple linear regression, it was decided how many predictors to be put in multiple linear regression. For each extra predictor, approximately 10 more samples were needed. Assuming 3 predictors entering the final model, *77* samples were considered to be enough.

2.7. Data Analysis

The Statistical Package for the Social Sciences (SPSS, IBM Corp. Version 25.0, NY, USA) was used to perform the analyses. The descriptive analysis was reported for the continuous data by mean and standard deviation and for the discrete data by frequency and percentage. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to verify the normality of the collected data. Four outcome variables were investigated namely PR, RR, SpO_2 (scale), and behavior (ordinal). Also, there were five predictors in this study namely age, parenting style, anxiety, treatment duration (scale), and sex (categorical). A *P* value of less than 0.05 was considered to be statistically significant.

To evaluate the association of each predictor and outcome measure (total score), simple linear and ordinal regressions were conducted. Those predictors that had $P<0.2$ entered into the multiple regression models. The normal distribution of the residuals was controlled by the P-P plots, and multicollinearity was checked using the variance inflation factor (VIF). Since there was high collinearity between mother's and father's parenting style, only the mother's parenting style was entered into the models. Further, SCAS and PAS scores were combined into a single variable using standardization by converting them into a Z score.

The repeated measure ANOVA and Friedman tests were used to compare the results between the five time points. If the global tests were significant, post hoc tests with Bonferroni correction would run. General Estimation Equation (GEE), which is a quasi-likelihood method was used to evaluate the predictive value of each independent variable considering the data of all the five time points. Using GEE, samples in each cluster (time point) were added together and the model ran with 400 samples. The GEE was applied for two different types of analyses. In the first analysis, treatment duration which was used as a predictor in regression models was substituted with time point (T1-T5) with five levels. Therefore, we could identify the effect of each condition related to each time point on the outcome measures. In the second analysis, GEE was used to analyze the relationship between behaviors and vital signs throughout the five time-points considering the predictors which were significant in the first analysis.

3. RESULTS

3.1. Descriptive Analyses

3.1.1. Independent variables

Eighty children with an average age of 7.20±2.22 years participated in this study. Forty children were female and the other 40 participants were male. The mean treatment duration was 30.15±10.84 minutes. The mean anxiety score was 19.71±10.98 for pre-school children and 16.07±10.54 for school-aged children. Among mothers and fathers, the authoritative parenting style was dominant, 3.99±0.58 and 3.89±0.60, respectively. The detailed analyses of the independent variables are shown in Table 1.

Table 1. ***Descriptive analysis of independent variables***

		Continuous Data			
		Minimum	Maximum	Mean	SD
Age (year)		3	12	7.20	2.22
Anxiety	*Preschool*	3	43	19.71	10.98
	School	1	48	16.07	10.54
Treatment Duration (minutes)		13	61	30.50	10.84
Parenting Style	*Authoritarian (M)*	1.08	2.75	1.78	0.36
	Authoritative (M)	1.80	5.00	3.99	0.58
	Permissive (M)	1.20	4.00	2.23	0.59
	Authoritarian (F)	1.00	3.25	1.76	0.38
	Authoritative (F)	1.80	4.93	3.89	0.60
	Permissive (F)	1.20	3.60	2.17	0.55
		Discrete Data			
		Frequency	Percentage	Cumulative Percentage	
Sex	*Female*	40	50	50	
	Male	40	50	100	
Parenting style	*Authoritarian (M)*	0	0	0	
	Authoritative (M)	76	95	100	
	Permissive (M)	4	5	4	
	Authoritarian (F)	0	0	0	
	Authoritative (F)	76	95	100	
	Permissive (F)	4	5	4	

Abbreviations
SD: standard deviation; M: mother; F: father

3.1.2. Dependent Variables

The total PR was 77.60±8.84 per minute. The highest and lowest mean for PR was seen in T3 (80.13±9.73) and T2 (75.74±8.60) respectively. The total RR was 23.56±1.81 per minute. The highest and lowest mean for RR was seen in T2 (23.88±3.07) and T1 (22.68±3.52) respectively. The total SpO_2 was 97.86±0.86 percent. The highest and lowest mean for SpO_2 was seen in T5 (97.93±.95) and T3 (97.34±1.59) respectively. In our study, the SpO_2 dropped more than 5% only in 2 patients, both between T2 and T3, which corresponded with their movement as recorded by the pulse oximeter. Regarding the behaviors, considering the whole procedure, 51 children showed an F-Score of 4 (63.7%), 23 children F-Score of 3 (28.7%), and 6 children F-Score of 2 (7.5%), and no child had the F-Score of 1. The best behavior was seen in T2 in which 62 (77.5%) had the F-Score of 4, and the worst behavior was seen in T3 with only 44 (55.0%) children showed an F-Score of 4. The detailed analyses of the dependent variables are shown in Table 2.

Table 2. ***Descriptive analysis of dependent variables***

		Continuous Data			
		Minimum	Maximum	Mean	SD
PR	*T1*	60	100	78.05	8.90
	T2	57	100	75.74	8.60
	T3	63	107	80.13	9.73
	T4	60	102	77.86	9.27
	T5	61	104	79.09	8.60
	Total	63	101	77.60	8.84
RR	*T1*	14	32	22.68	3.52
	T2	16	30	23.88	3.07
	T3	16	34	23.74	3.35
	T4	16	32	23.71	2.90
	T5	18	32	23.77	2.79
	Total	19	28	23.56	1.81
SpO_2	*T1*	90	99	97.41	1.28
	T2	90	99	97.48	1.24
	T3	90	100	97.34	1.59
	T4	90	100	97.88	1.23
	T5	95	100	97.93	0.95

	Total		95	99	97.86	0.86
Discrete Data						
			Frequency		Percentage	Cumulative Percentage
Behavior	*T1*	*1*	0		0	0
		2	6		7.5	7.5
		3	28		35.0	42.5
		4	46		57.5	100
	T2	*1*	0		0	0
		2	1		1.3	1.3
		3	17		21.3	22.5
		4	62		77.5	100
	T3	*1*	1		1.3	1.3
		2	5		6.3	7.5
		3	30		37.5	45.0
		4	44		55.0	100
	T4	*1*	0		0	0
		2	7		8.8	8.8
		3	14		17.5	26.3
		4	59		73.8	100
	T5	*1*	0		0	0
		2	8		10.0	10.0
		3	22		27.5	37.5
		4	50		62.5	100
	Total	*1*	0		0	0
		2	6		7.5	7.5
		3	23		28.7	36.3
		4	51		63.7	100

Abbreviations
SD: standard deviation; PR: pulse rate; RR: respiratory rate; SpO_2: oxygen saturation

3.2. Inferential Analyses

3.2.1. Regression Analyses

3.2.1.1. Simple Regressions

The simple linear regressions were conducted for the total scores of PR, RR, SpO_2, and ordinal regression for the total score of behavior to identify the eligible predictors ($P<0.20$) to be entered into multiple regression models. Regarding PR, age (B=-1.83, P<0.001), sex (B=4.25, P=0.03), and treatment duration (B=-0.13, P=0.14) were eligible. Regarding the RR, age (P=0.001, B=-.02) and treatment duration (B=-0.04, P=0.009) were eligible. Regarding the behaviors, only sex (odds ratio [OR]=2.31, P=0.07) was eligible, therefore multiple ordinal regression was not performed. Lastly, none of the predictors was significant for the SpO_2.

3.2.1.2 Multiple Regressions

After running the multiple linear regression for PR, only age was significant (B=-1.73, P<0.001), and sex and treatment duration lost their significance (P>0.05). Age also significantly predicted RR (B=-0.24, P=0.01) while treatment duration lost its significance (P>0.05).

Also, multiple ordinal regression was run to test the relationship between vital signs in the five time points with the behaviors in the five time points. Therefore 25 different tests were run to investigate the relationship between each of the vital signs with behaviors. The only highly significant relationship was seen between PR in T2 (after administrating N_2O) with behaviors in T4 (during dental treatment), (OR=0.65, P=0.006).

3.2.2. Repeated Measure Analyses

3.2.2.1. Repeated Measure ANOVA and Post Hoc Test

The fluctuations of vital signs for PR (Fig. 3), RR (Fig. 4), and SpO_2 (Fig. 5) are depicted.

Figure 3. **The changes in pulse rate through the study time points**

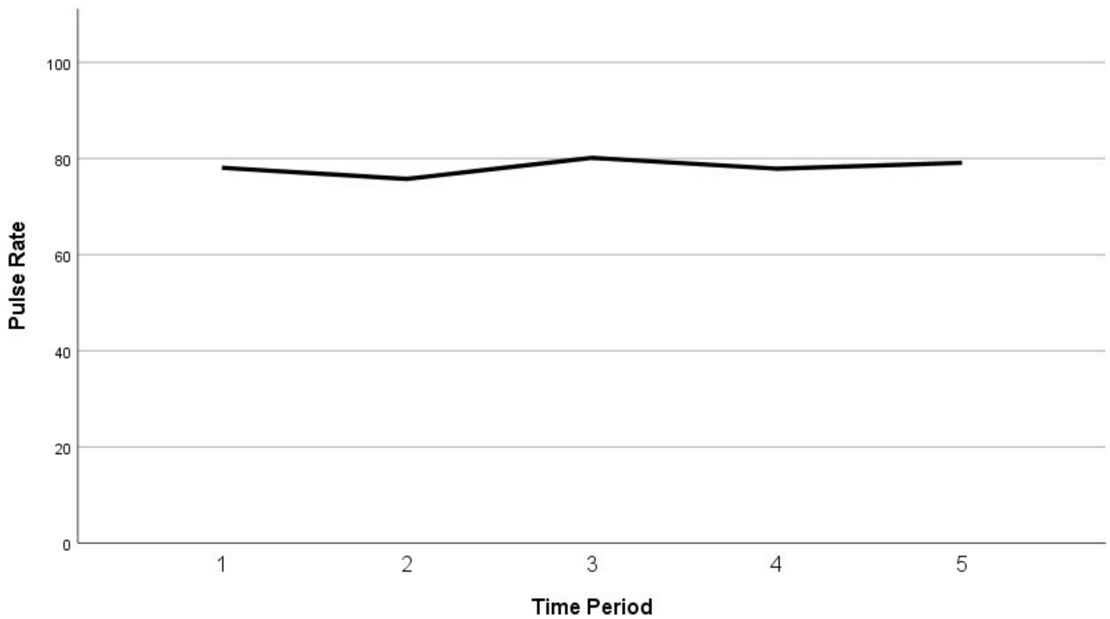


Figure 4. **The changes in respiratory rate through the study time points**

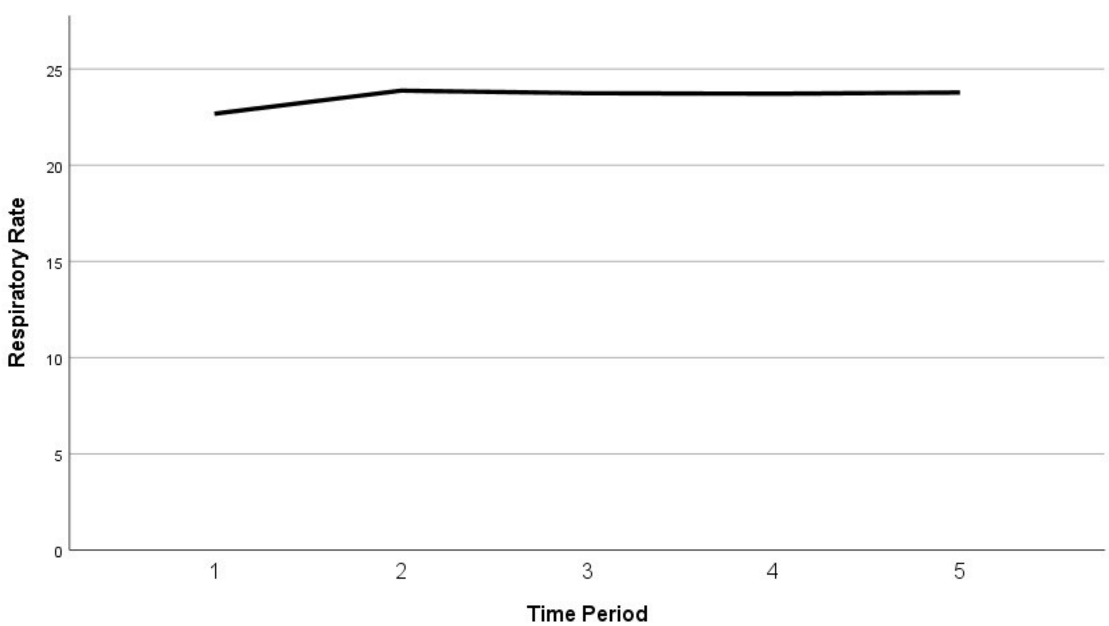
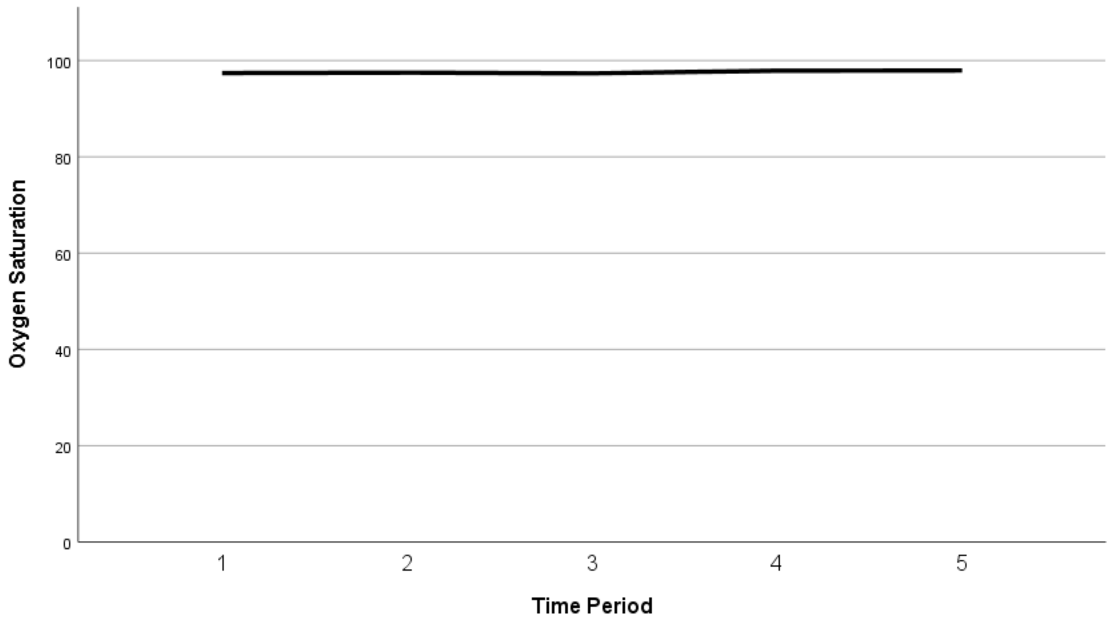


Figure 5. **The changes in respiratory rate through the study time points**



The repeated measure ANOVA was run for PR, RR, and SpO_2 considering data from the five time points (T1 to T5). Since the sphericity assumption was violated based on the Mauchly's test ($P<0.05$), the Greenhouse-Geisser correction was used to report the results. Based on the results, the PR [$F(3.05, 241.06) = 14.49$, $P<0.001$] and SpO_2 [$F (3.07, 242.53) = 4.74$, $P=0.003$] were significantly different between the time points. Also, regarding the RR, the differences were almost significant [$F (3.18, 251.18) = 2.39$, $P=0.06$]. Therefore, the post hoc tests with Bonferroni correction were run to determine which pairs of time points were significantly different for PR (Table 3), RR (Table 4), and SpO_2 (Table 5).

Table 3. ***Post hoc tests to determine differences in pulse rate between the time points of study***

Reference point (I)	Factor (J)	Mean difference (I-J)	Standard Error	P-Value
T1	T2	2.31	0.37	<0.01
	T3	-2.07	0.64	0.01
	T4	0.18	0.69	1.00
	T5	-1.03	0.64	1.00
T2	T3	-4.38	0.57	<0.01
	T4	-2.12	0.65	0.01
	T5	-3.35	0.59	<0.01
T3	T4	0.62	0.00	0.62
	T5	0.69	1.00	0.69
T4	T5	1.22	0.50	0.17

Table 4. ***Post hoc tests to determine differences in respiratory rate between the time points of study***

Reference point (I)	Factor (J)	Mean difference (I-J)	Standard Error	P-Value
T1	T2	-1.20	0.40	0.03
	T3	-1.06	0.47	0.29
	T4	-1.03	0.50	0.43
	T5	-1.10	0.48	0.26
T2	T3	0.13	0.38	1.00
	T4	0.16	0.49	1.00
	T5	0.10	0.44	1.00
T3	T4	0.02	0.49	1.00
	T5	-0.03	0.48	1.00
T4	T5	-0.06	0.31	1.00

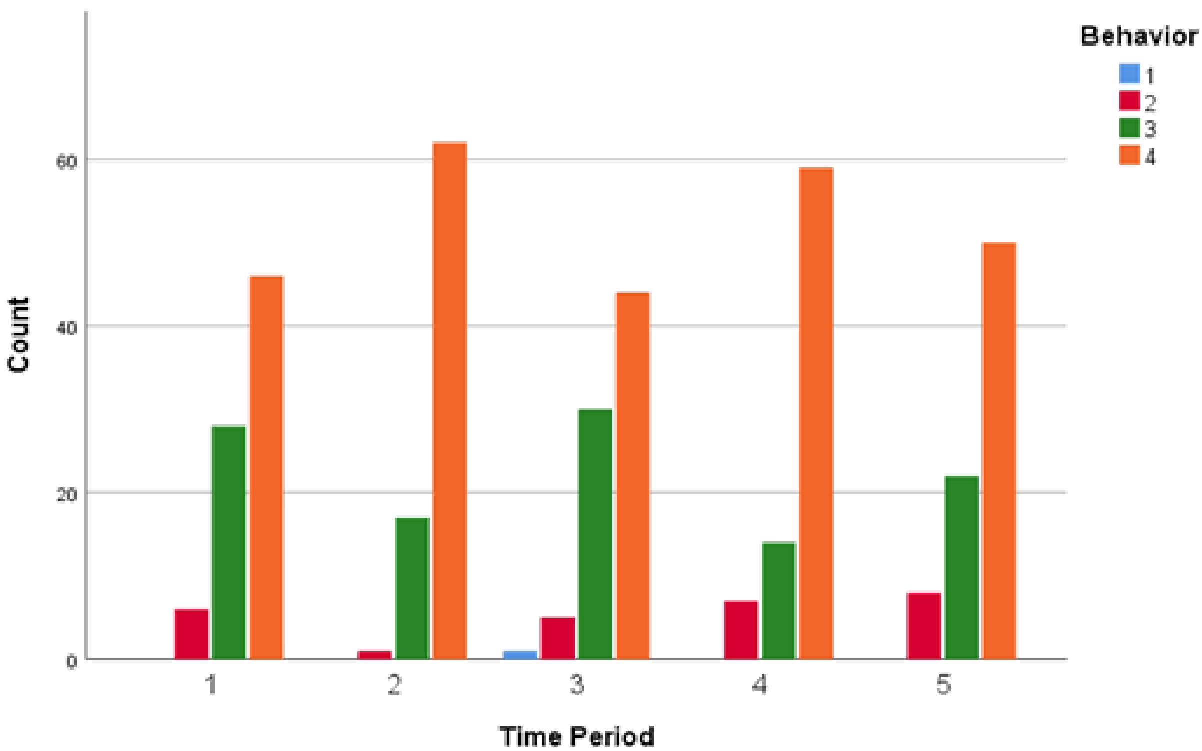
Table 5. **Post hoc tests to determine differences in oxygen saturation between the time points of study**

Reference point (I)	Factor (J)	Mean difference (I-J)	Standard Error	P-Value
T1	T2	-0.06	0.11	1.00
	T3	0.07	0.22	1.00
	T4	-0.46	0.18	0.14
	T5	-0.51	0.16	0.02
T2	T3	0.13	0.20	1.00
	T4	-0.40	0.17	0.24
	T5	-0.45	0.14	0.03
T3	T4	-0.53	0.20	0.08
	T5	-0.58	0.20	0.04
T4	T5	-0.05	0.13	1.00

3.2.2.2. Friedman and Wilcoxon tests

The changes in behaviors pertaining to each time point of the study are shown in the figure below (Fig. 6) The Friedman test was run for behaviors considering data from the five time points (T1 to T5). There was a statistically significant difference between time points regarding the behavior of children χ^2 (2)=20.31, P<0.001. Post hoc analysis using Wilcoxon signed-rank tests showed that there was a significant difference between behaviors in T1 and T2 (Z=-3.54, P<0.001), T2 and T3 (Z=-4.53, P<0.001), T2 and T5 (Z=-2.90, P=0.004), and T3 and T4 (Z=-2.04, P=0.04).

Figure 6. **The behaviors of children at each time point of the study**



3.2.3. General Equation Estimation (GEE)

3.2.3.1. Association between the Time Points and Outcome Measures

The GEE analysis was used to determine if T2, T3, T4, and T5 in comparison to T1 (reference point) could predict the outcome measures considering the possible role of other predictors. The linear model was run for PR, RR, and SpO_2. Regarding PR, result showed that age (B=-1.38, P=0.002), T3 (B=2.07, P=0.001), and T2 (B=-2.31, P<0.001) were significant (Table 6).

Table 6. **General Estimation Equation based on linear model to determine predictors of pulse rate**

Parameter	B	SE	95% Wald CI		Hypothesis Test			Odds Ratio
			Lower	Upper	Wald $\chi 2$	df	P-Value	
(Intercept)	94.66	10.10	74.8	114.46	87.78	1	<0.001	1.29E+41
Sex=Female	1.00	1.82	-2.56	4.58	0.30	1	0.58	2.740
Sex=Male	0[a]	.	.	.	.	.	.	1
Time point (T5)	1.03	0.63	-0.21	2.28	2.64	1	0.10	2.82
Time point (T4)	-0.18	0.69	-1.54	1.17	0.07	1	0.78	0.82
Time point (T3)	2.07	0.63	0.82	3.32	10.57	1	0.001	7.96
Time point (T2)	-2.31	0.37	-3.04	-1.58	38.40	1	<0.001	0.09
Time point (T1)	0[a]	.	.	.	.	.	.	1
Age	-1.38	0.44	-2.26	-0.51	9.65	1	0.002	0.24
Parenting Style (Permissive)	-1.97	1.39	-4.71	0.75	2.00	1	0.15	0.13
Parenting Style (Authoritarian)	-3.36	2.20	-7.69	0.96	2.32	1	0.12	0.03
Parenting Style (Authoritative)	0.82	1.40	-1.92	3.57	0.34	1	0.55	2.27
Anxiety	0.69	0.85	-0.97	2.36	0.65	1	0.41	1.99

Abbreviations
[a] reference category; SE: standard error; CI: confidence interval; χ2: chi-square; df: degree of freedom

Regarding RR, T5 (B=1.10, P=0.02), T4 (B=1.03, P=0.03), and T3 (B=1.06, P=0.02) were almost significant, and T2 (B=1.20, P=0.003) and age (B=-0.26, P=0.003) were highly significant (Table 7). Regarding SpO_2, only T5 (B=0.52, P=0.001) and T4 (B=0.47, P=0.01) were significant (Table 8).

Table 7. **General Estimation Equation based on linear model to determine predictors of respiratory rate**

Parameter	B	SE	95% Wald CI		Hypothesis Test			Odds
			Lower	Upper	Wald $\chi2$	df	P-Value	Ratio
(Intercept)	23.46	2.50	18.56	28.36	88.06	1	<0.001	0.15B
Sex=Female	-0.08	0.40	-0.89	0.71	0.04	1	0.82	0.91
Sex=Male	0[a]	.	.	.	.	.	.	1
Time point (T5)	1.10	0.48	0.15	2.04	5.17	1	0.02	3.00
Time point (T4)	1.03	0.50	0.05	2.02	4.25	1	0.03	2.82
Time point (T3)	1.06	0.47	0.12	1.99	4.98	1	0.02	2.89
Time point (T2)	1.20	0.39	0.42	1.98	9.10	1	0.003	3.32
Time point (T1)	0[a]	.	.	.	.	.	.	1
Age	-0.26	0.08	-0.44	-0.08	8.71	1	0.003	0.76
Parenting Style (Permissive)	-0.42	0.38	-1.17	0.33	1.20	1	0.27	0.65
Parenting Style (Authoritarian)	0.48	0.55	-0.60	1.56	0.75	1	0.38	1.61
Parenting Style (Authoritative)	0.31	0.48	-0.63	1.25	0.42	1	0.51	1.36
Anxiety	0.08	0.23	-0.38	0.54	0.11	1	0.73	1.08

Abbreviations
[a] reference category; SE: standard error; CI: confidence interval; $\chi2$: chi-square; df: degree of freedom

Table 8. **General Estimation Equation based on linear model to determine predictors of oxygen saturation**

Parameter	B	SE	95% Wald CI		Hypothesis Test			Odds Ratio
			Lower	Upper	Wald χ2	df	P-Value	
(Intercept)	98.30	1.21	95.93	100.67	6595.58	1	<0.001	4.93E+42
Sex=Female	0.01	0.156	-0.28	0.32	0.01	1	0.91	1.01
Sex=Male	0[a]	.	.	.	.	.	.	1
Time point (T5)	0.52	0.16	0.21	0.83	10.75	1	0.001	1.69
Time point (T4)	0.47	0.18	0.11	0.83	6.68	1	0.01	1.60
Time point (T3)	-0.06	0.21	-0.49	0.36	0.08	1	0.77	0.93
Time point (T2)	0.07	0.11	-0.15	0.30	0.40	1	0.52	1.07
Time point (T1)	0[a]	.	.	.	.	.	.	1
Age	0.01	0.04	-0.07	0.10	0.13	1	0.71	1.01
Parenting Style (Permissive)	0.00	0.10	-0.19	0.20	0.00	1	0.97	1.00
Parenting Style (Authoritarian)	-0.11	0.28	-0.67	0.44	0.17	1	0.67	0.88
Parenting Style (Authoritative)	-0.20	0.17	-0.54	0.13	1.42	1	0.23	0.81
Anxiety	-0.05	0.08	-0.23	0.11	0.40	1	0.52	0.94

Abbreviations
[a] reference category; SE: standard error; CI: confidence interval; χ2: chi-square; df: degree of freedom

The ordinal logistic model was run for the behaviors. Based on the results, T2 (OR=2.69, P=0.001) and the authoritative parenting style (OR=1.93, P=0.01) significantly positively associated with the behaviors of children (Table 9).

Table 9. **General Estimation Equation based on the ordinal model to determine predictors of behaviors**

Parameter		B	SE	95% Wald CI		Hypothesis Test			Odds
				Lower	Upper	Wald $\chi 2$	df	P-Value	Ratio
Threshold	***[Behavior=2]***	1.25	1.62	-1.94	4.44	0.59	1	0.44	3.50
	[Behavior=3]	3.36	1.61	0.20	6.51	4.35	1	0.03	28.81
Sex=Female		-0.52	0.35	-1.22	0.16	2.21	1	0.13	0.59
Sex=Male		0[a]	.	.	.	.	.	.	1
Time point (T5)		0.11	0.26	-0.40	0.64	0.19	1	0.66	1.12
Time point (T4)		0.66	0.34	-0.01	1.34	3.68	1	0.05	1.94
Time point (T3)		-0.06	0.24	-0.54	0.41	0.08	1	0.77	0.93
Time point (T2)		0.99	0.28	0.43	1.55	12.04	1	0.001	2.69
Time point (T1)		0[a]	.	.	.	.	.	.	1
Age		0.01	0.06	-0.11	0.14	0.03	1	0.84	1.01
Parenting Style (Permissive)		0.06	0.27	-0.46	0.59	0.05	1	0.80	1.06
Parenting Style (Authoritarian)		0.61	0.45	-0.27	1.50	1.84	1	0.17	1.85
Parenting Style (Authoritative)		0.66	0.27	0.12	1.20	5.74	1	0.01	1.93
Anxiety		-.11	0.17	-0.46	0.24	0.37	1	0.54	0.89

Abbreviations
[a] reference category; SE: standard error; CI: confidence interval; $\chi 2$: chi-square; df: degree of freedom

3.2.3.2. Association between Behaviors and Vital Signs

The association between vital signs and the behaviors of children is depicted for PR (Fig. 6), RR (Fig. 7), and SpO_2 (Fig. 8). As it is seen in the figures, no meaningful trend can be drawn for any of the vital signs and behaviors meaning that an increase or a decrease in vital signs does not seem to be related to behaviors of children through the time points of the study.

***Figure 7*. The association between pulse rate and behaviors for the five time points of study**

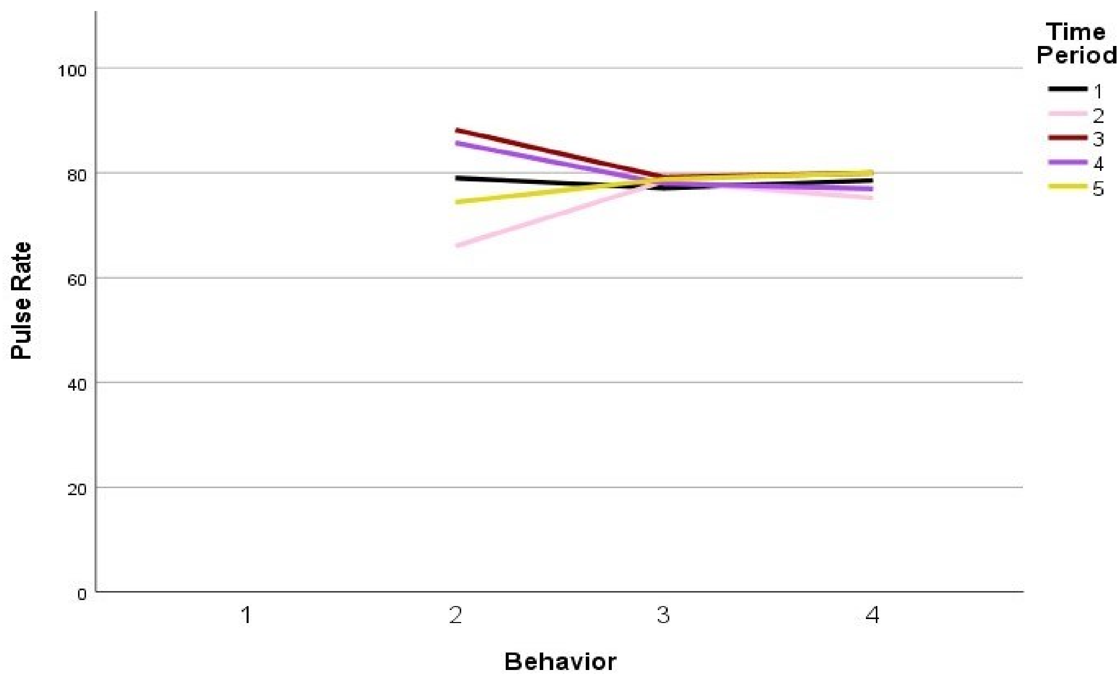


Figure 8. **The association between respiratory rate and behaviors for the five time points of study**

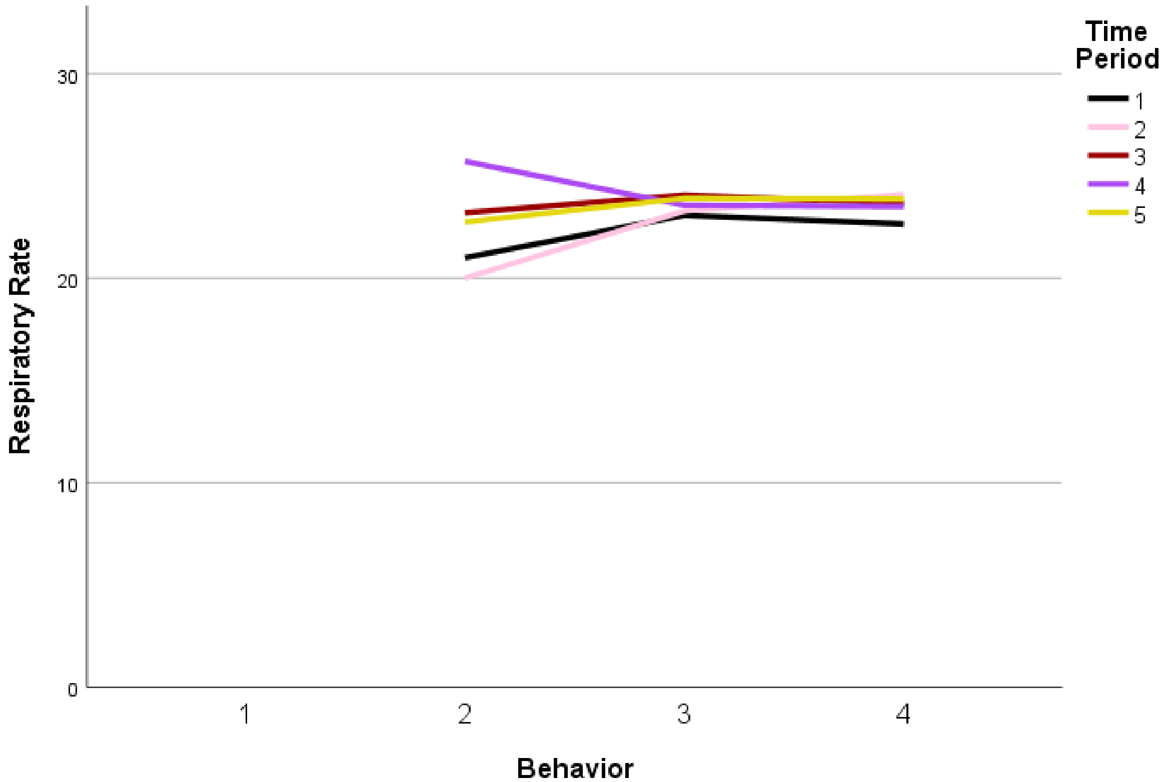
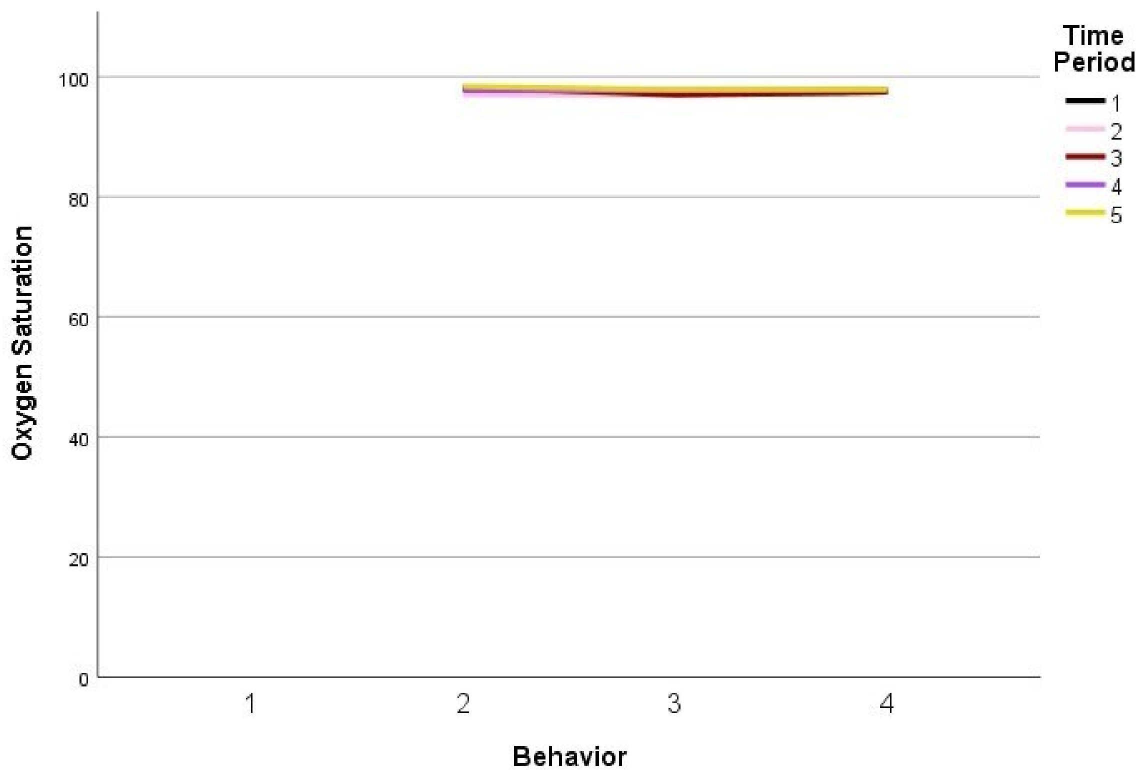


Figure 9. **The association between oxygen saturation and behaviors for the five time points of study**



To determine the real-time association of vital signs and behaviors, The GEE was run considering behaviors as the outcome measure to evaluate if PR, RR, or SpO_2 can predict behaviors. Time points (T1 to T5) and age which were important predictors of behaviors and vital signs were also entered into the model as confounders. The results showed that there was no association between behaviors and any of the vital signs (Table 10).

Table 10. **General Estimation Equation based on the ordinal model to determine the association between behaviors and vital signs**

Parameter		B	SE	95% Wald CI		Hypothesis Test			Odds Ratio
				Lower	Upper	Wald $\chi2$	df	P-Value	
Threshold	***[Behavior=2]***	5.35	8.53	-11.37	22.08	0.39	1	0.53	211.33
	[Behavior=3]	7.38	8.57	-9.42	24.18	0.74	1	0.38	1605.14
Time point (T5)		0.09	0.26	-0.41	0.61	0.13	1	0.71	1.10
Time point (T4)		0.59	0.34	-0.07	1.26	3.00	1	0.08	1.80
Time point (T3)		-0.03	0.23	-0.49	0.42	0.02	1	0.87	0.96
Time point (T2)		0.90	0.28	0.35	1.46	10.20	1	0.001	2.48
Time point (T1)		0[a]	.	.	.	.	.	.	1
Age		0.01	0.07	-0.12	0.14	0.02	1	0.88	1.01
PR		0.01	0.03	-0.06	0.09	0.13	1	0.71	1.01
RR		-0.00	0.01	-0.04	0.02	0.14	1	0.69	0.99
SpO_2		0.08	0.08	-0.08	0.24	0.90	1	0.34	1.08

Abbreviations
[a] reference category; SE: standard error; CI: confidence interval; $\chi2$: chi-square; df: degree of freedom; PR: pulse rare; RR: respiratory rate; SpO_2: oxygen saturation

4. DISCUSSION

4.1. Summary of the Results

The results of the current study showed that, statistically, N_2O/O_2 led to a decrease in PR, an increase in RR, insignificant changes in SpO_2, and better behaviors. However, the changes were clinically negligible meaning that the scores obtained for the vital signs were in a clinically acceptable range and considered safe. During the moderate sedation by N_2O/O_2, the dental injection had the most impact on vital signs and behaviors by increasing PR and worsening the behaviors. The dental treatment itself did not change behaviors and vital signs significantly. Switching from 40% N_2O/O_2 to 100% O_2 at the end of the procedure did not make a significant change in any of the vital signs nor the behaviors. In terms of the predictors, age inversely associated with PR and RR but did not correlate with SpO_2 and behaviors. The authoritative parenting style predicted the cooperative behavior of children, but except for that, other predictors including sex, anxiety, and treatment duration did not predict vital signs or behaviors. Furthermore, a significant real-time association was not found between vital signs and behaviors.

4.2. Interpreting the Results

Some points should be taken into account when interpreting the results of this study.

- *First,* unlike some previous reports, audio-visual distraction was used in this dental practice as part of their routine care, which has been shown to reduce anxiety, promote cooperative behaviors during the injection, and improve constructive behavioral response after the dental injection. Audio-visual distraction was also shown to lower PR in children undergoing dental treatments (95–97). The importance of audio-visual distraction was also emphasized in the medical field in reducing pain and distress for children undergoing painful procedures (98).

- *Second,* the local anesthesia was administrated using a controlled local anesthetic delivery system which decreases the pain and improves cooperative behavior compared with cartridge syringe in children (99,100). Therefore, less excitatory stimuli were perceived by children which might have caused less arousal and valence by ANS (60,63).
- *Third,* the behaviors of children were scored using live observation rather than going through the video and audio recordings. Live observation suits the behavioral scale used in this study since simple coding systems (e.g. Frankl scale) are more consistent with live observation, while recording is more appropriate for more complex coding systems (101).
- *Fourth,* using a pulse oximeter, the peripheral oxygen saturation in the blood (SpO_2) can be measured which almost but not always is similar to arterial oxygen saturation (SaO_2). SpO_2 and SaO_2 are reliably correlated except in young children and seriously ill patients (102).
- *Fifth,* in our study, children were supine and stimulated not only by voice and face mask but also with audio-visual distraction, mouth isolation, local injections, and parents' communication in some cases. These various external stimuli might have ultimately impacted the child's vital signs, behaviors, and level of consciousness (100).

4.3. Phases of the Dental Procedure

We compare our results with the findings of previous reports in three different conditions pertaining to the different time points of our study discussed earlier.

Condition 1 (T1 to T2) evaluates the effect of N_2O itself on the vital signs and behaviors. The vital signs including PR, RR, and SpO_2 were recorded before administrating the N_2O and taken as base values. After exposing participants to N_2O, vital signs and behaviors were measured again, and as a result, the changes were attributed to the gas effect.

Condition 2 (T2 to T4) evaluates the effect of dental injections and dental treatments on the vital signs and behaviors. Since the CNS shows reaction to dental treatment, the effect of N_2O can be masked and moderated by different stimuli during the treatment (103). It has been shown that in patients who are not going under moderate sedation, stress and fear during the treatment can significantly impact the physiological responses (104). For example, a higher RR can be expected during dental treatments due to obstructive effects, stress, anxiety, and possibly inadequate anesthesia (105). The PR and RR have been shown to increase in the dental environment due to fear and anxiety. The increased PR in dental settings can be considered a normal physiological response to stressful dental procedures and should be differentiated from the gas effect (106,107). Moreover, different types of treatments have different impacts on physiological responses; For example, exodontia causes significant fluctuations in physiological responses while restorative treatment leads to limited fluctuations (104,105).

Condition 3 (T4 to T5) evaluated the effect of 100% O_2 on vital signs and behaviors when N_2O was discontinued. The main reason to provide 100% O_2 after N_2O is to avoid hypoxia due to discontinuation of N_2O, but it is important to know how the transition affects behaviors and other vital signs besides SpO_2. Previous studies used the control trial design and compared the effect of N_2O and O_2 in different groups and not across a single treatment session. Our within-subject design let us evaluate how transitioning from N_2O to O_2 in a real dental setting affect vital signs and behaviors.

4.4. Fluctuations in Vital Signs and Behaviors

Based on the classifications of the dental procedure described above, we compared our results with similar studies in terms of changes in vital signs and behaviors for each condition.

4.4.1. Pulse Rate

Condition 1 (T1 to T2): Our results showed that N_2O decreased PR from 78.05±8.90 to 75.74±8.60 which was statistically significant but clinically negligible. Bonafe-Monzó et al. showed that in participants with a mean age of 22.45 ± 3.53 years, PR decreased after administration of 50% N_2O (70.21±12.50 vs. 67.59±10.41) (53). Moreover, in Leelataweewud's study, patients with the mean age 41 months who inhaled 40% N_2O (premediated by midazolam) had a lower heart rate compared to the baseline (104±2.6 vs. 121±19.8) (93). On the contrary, the study by Alzahrani showed that adding 30-50% N_2O to oral midazolam anesthetic regimen of children with an average age of 55.07±9.29 months did not change the PR significantly (108).

Condition 2 (T2 to T4): In our study PR increased during the dental injection (80.13±9.73) and gradually decreased during the treatment (77.86±9.27). The same trend was seen in the study by McCann who used 50% N_2O (premeditated by chloral hydrate and hydroxyzine). McCann also noticed that in patients undergoing 100% O_2, PR follows the same but the weaker trend (109).

Conditions 3 (T4 to T5): In our study, PR increased non-significantly after switching from N_2O to O_2. Two previous reports with crossover trial designs also showed that although PR was lower in N_2O compared to O_2, the differences were not significant (93,109). However, another study showed that the differences were significant and PR was higher in the O_2 group (89±0.6 vs. 87±0.5) (51). Similar to our study, Bonafe-Monzó et al. implemented a within-subject design meaning administrating O_2 following N_2O and found that PR decreased (67.59±10.41 vs. 65.63±11.39).

However, the participants in Bonafe-Monzó's study were young adults, and measurements did not take place in dental settings (53).

Summary: N_2O has partial cardiovascular effects due to myocardial contractility depression (23–25). Besides the gas effect, other factors may play a role in fluctuations of PR as well which are unrelated to the pharmacologic effect of N_2O. Possibly, a part of observed changes in PR can be due to greater patient relaxation and sporadic movements (110).

4.4.2. Respiratory System

Condition 1 (T1 to T2): Similar to PR, changes in RR were clinically negligible but statistically significant. The RR increased from 22.68±3.52 to 23.88±3.07 after administrating the N_2O which agrees with the result of previous reports. Primosch showed that in children with the mean age of 7.3 years, 40% N_2O caused a slight increase in RR (22.27±0.31 vs. 22.05±1.05) (52). In a study by Litman, children with a mean age of 3.8 years undergoing 40% N_2O (premeditated with midazolam) had higher RR compared to the baseline (28.3±6.8 vs. 32.9±7.1) (111); the same results were seen for 50% N_2O premediated by chloral hydrate (112). Litman also showed that 60% N_2O increased the RR more than 15% N_2O in 1-3-year-old children premeditated with midazolam (57). The reason behind the increase in RR may be due to the respiratory stimulant effect of N_2O leading to an increase in sympathetic tone. That being said, although statistically significant, the increase in RR cannot be considered clinically meaningful. Unlike RR, results regarding the effect of N_2O on SpO_2 was not consistent. Similar to some studies (57,111,112), our results showed no significant changes in SpO_2. However, some other studies showed that oxygen level increased after administration of N_2O (99.25±0.55 vs. 98.97±0.59) (53), (98±0.2 vs. 97±1.0) (93), and (98.53±0.78 vs. 97.60±1.0) (108) probably due to the availability of much higher oxygen concentration than atmospheric air.

Condition 2 (T2 to T4): Only negligible differences were seen in our study when transitioning from N_2O to dental injection regarding RR and SpO_2. Also, moving from dental injection to dental treatment, RR did not change but SpO_2 increased probably due to children being more relaxed. Primosch study reported a small increase in RR during the dental injection (23.35±0.60) and restorative treatment (22.66±0.24) compared to the before treatment (22.27±0.31) (51). Unlike our results, McCann reported that SpO_2 decreased during the dental injection (109) probably due to movements and false reading of the pulse oximeter. Because oxygen level normally fluctuates in a limited range, significant changes can be associated with conditions unrelated to the pharmacological effect such as the patients' movement and crying (107,113).

Conditions 3 (T4 to T5): Our results showed that transitioning from N_2O to O_2 did not cause a significant change to RR or SpO_2. A previous study showed that PR and RR did not differ significantly between individuals receiving supplemental oxygen and those who breathed room air. The only difference was that individuals receiving supplemental oxygen had a higher SpO_2 which helped to prevent hypoxia (114). The results of previous reports were not consistent regarding RR and SpO_2. While one study showed that RR was higher in the N_2O group than the O_2 group (21±4.7 vs. 20±4.7) (93) another study showed the opposite (22.27±0.31 vs. 23.25±0.28) (52). Also, compared with O_2, N_2O either did not change SpO_2 (93,109) or increased it (99.04±0.62 vs. 98.97±0.59) (53).

Summary: One of the main complications of using anesthetic agents during dental procedures is respiratory depression. The manifestation of respiratory depression is a decrease in respiratory drive and inability to maintain the upper airway. Respiratory depression can lead to dangerous conditions such as hypoxemia (115) which is defined as a drop in SpO_2 of 5% from baseline (93,116). In our study, the SpO_2 dropped only in 2 patients which corresponded with their movement as recorded by the pulse oximeter, and as a result, was not related to the pharmacological effect of N_2O (107,113). Except for those 2 instances, the dental procedure led to a mild increase in SpO_2 which can be explained by the mild increase in the respiratory rate (105). Similar to our results, Verwest et al. showed that 40% N_2O did not lead to hypoxemia in any of the patients (113). The concurrent use of O_2 with N_2O is probably the reason behind not developing hypoxemia (117). The beneficial use of supplemental oxygen administration concurrent with inhalation anesthetic is supported by both animal (118) and human (119,120) studies.

4.4.3. Behaviors

Condition 1 (T1 to T2): Our results showed that after administrating the N_2O, the number of children with definitely-positive behaviors increased from 57.5% to 77.5%. No other study reported on behaviors before and after administrating N_2O or between patients undergoing N_2O and those who were not.

Condition 2 (T2 to T4): Our results were consistent with previous reports and showed the worst behavior was expressed during the dental injection and was reversed and followed by more cooperative behavior during the dental treatment. Similar to our study, two other reports that used the Ohio State University Behavior Rating Scale (OSUBRS), showed the same trend of behaviors. The rate of crying, struggling, and movement increased while the rate being quiet decreased during the dental injection; the opposite trend was seen during the dental treatment (109,121).

Conditions 3 (T4 to T5): Our results showed that behaviors of children worsened switching from N_2O to O_2 but non-significantly. McCann using OSUBRS showed that there were no significant differences between N_2O and O_2 although the children were more cooperative under N_2O (109).

Also, Wilson and Primosch showed that, compared to O_2, N_2O reduced crying and struggling while increased quiet behaviors significantly. There was no interaction between the effect of gas and procedure on the behaviors (51,121).

Summary: More studies are needed to identify the effect of N_2O on the behaviors. Regarding the behaviors during dental treatment, although previous studies used a different instrument (OSUBRS) than the Frankl scale used in our study, the results were consistent. Behaviors of children worsen even when the injection was delivered using a controlled local anesthetic delivery under moderate sedation. Apart from the injection, the treatment itself did not seem to affect the behaviors meaningfully.

4.5. Effect of Predictors

There are some reports in the literature on the role of moderators and confounders related to behaviors and physiological responses. However, these reports did not evaluate the effects of demographics, treatment duration, anxiety, and parenting style on children and adults who received N_2O and underwent moderate sedation. Regarding the demographics, the reason for lack of evaluation is probably due to implementing the crossover study design in which researchers matched age and sex. The within-subject design of our study let us measure the effect of demographics and other predictors. In our study, we focused on some important predictors to have a better understanding of the effect of N_2O in order to avoid generalizability. Therefore, it will be elucidated for which group of patients N_2O is the most effective.

Our results showed that age inversely associated with PR and RR, but there was no interaction between age and any of the time points meaning that age, gas effect, and the treatment procedure had an independent effect on the PR and RR. The age range in our study was 2 to 12 years which is considered the rapid growth period. In this period, PR and RR naturally decrease when children get older. The change in values obtained for PR and RR in our study corresponded with the normal range and centiles of PR and RR in the literature (71–73). Adjusting the analyses on the age let us identify the true effect of other predictors.

There was no difference between males and females in this study, and no interaction was found between sex and any of the time points. Although there are differences between males and females in terms of PR and RR in the normal population (71,122), the magnitude of the difference is not the same in different age groups. The difference between males and females becomes more prominent after adolescence (122). Our results agree with the only report since 1996 that took into account the role of sex in predicting the effect of N_2O on the physiological responses. This study found no significant differences between males and females in terms of PR and SpO_2 (53).

Previously, a maximum treatment time of 15 minutes was suggested for children up to 5 years of age (123,124). A new study showed that treatment duration is age-dependent and can vary between 20 minutes for younger children to 60 minutes for older children before the behaviors worsen; however, this study did not make it clear if the treatment duration was measured for children undergoing moderate sedation by N_2O or other sedatives (125). In contrast to this report, our results showed no interaction between age and treatment duration. Also, the treatment duration did not associate with behaviors or physiological responses. The possible explanation for this discrepancy is that N_2O, to some extent, made the dental procedure more tolerable as it was seen with other sedative agents such as midazolam (126). Also, in the dental office where the treatment took place, all children were exposed to audio-visual distractions that have been shown to reduce anxiety and promote more cooperative behaviors (95,97). It should also be noted that the average treatment duration (T3 and T4) of our study was actually less than 30.50 minutes which is representative of the whole procedure (T1 to T5).

Regarding parenting style, the authoritative style was the most common type of relationship between children and their parents which also predicted better behaviors. Our results agree with several previous studies showing that the authoritative parenting style is associated with better behaviors of children in dental settings. However, it was not mentioned in those studies if children were under moderate sedation or not (79–81). Our results showed that regardless of children being sedated or not, parenting style plays an important role in the behaviors of children but not for physiological responses. These results agree with psychological research which emphasizes the importance of the immediate family environment on forming personality and behaviors. Specifically, it was shown that the authoritative child-parent relationship fosters happiness, emotional control, and better social skills (127–129). Also, in interpreting the results, it should be noted that parents in our study were present in the operatory room where the treatment took place, and they could communicate with their children during the treatment.

In our study, anxiety did not affect either the behaviors or vital signs which is in contrast with some previous studies that showed anxiety led to worse behaviors during dental treatments (76,77,82). The reasons behind our findings may be due to the fact that the anxiety score ranged from 1 to 48 out of the maximum possible score of 114. Also, the mean anxiety score was 19.71 for pre-school children 16.07 for school-age children. The possible reason for the low anxiety scores might have been due to the selection process for children undergoing dental treatment. Based on the decision of the clinicians, the uncooperative children in the examination day or those who showed uncooperative behaviors during the previous dental treatments were advised to consider general anesthesia. This cohort of patients might have had more anxiety compared to the patients who received treatments in the dental office. More importantly, since N_2O has anxiolytic besides analgesic and anesthetic effects, it possibly reduced the anxiety of patients during the dental procedure.

4.6. Association between Behaviors and Vital Signs

Our results showed that the high PR in T2 could predict poor behavior in T4. This information will be helpful for clinicians to decide on how to modify the plan and implement necessary behavioral management. Besides that, there was no association between the average scores for vital signs including PR, RR, and SpO_2 with behaviors meaning that physiological parameters cannot be predicted from behaviors and vice versa in real-time. This finding is important because it shows that by just observing children's cooperative behaviors in a dental office, one cannot say if their vital signs are stable as well. On the other hand, children's uncooperative behavior may not signal fluctuation in vital signs either. We found that the changes in vital signs reported in this study, although statistically significant, were in normal clinical range posing no threat to the health of children. However, the inconsistency between vital signs and behaviors may signal a different emotional status that needs further investigation. For example, while a child seems to be cooperative, higher scores of vital signs may indicate arousal and valence (activation) which are part of fundamental dimensions that organize the emotional responses (130–132). The valence dimension makes a distinction between states of pleasure (e.g., happy) and states of displeasure (e.g., sad). The arousal dimension makes a distinction between low excitement (e.g., quiet) and high excitement (e.g., surprised) (63). On the other hand, a child may have stable and normal vital signs, while his/her behavior shows some degree of emotion specify such as fear (63). Therefore, it is important to take into account both vital signs and behaviors in interpreting the emotions of children.

The results of our study are in contrast with two previous reports that used different sedative regimens. Wilson et al. found that when using 50% N_2O (premediated by chloral hydrate and hydroxyzine), the rate of crying and struggling increased corresponding to an increase in PR and blood pressure. On the other hand, the rate of quietness increased corresponding to a decrease in PR and blood pressure (121). Blumer et al. found that there was more fluctuation in oxygen saturation of children with poor behavior than good to excellent behaviors who were sedated with midazolam. The average oxygen saturation level was higher and PR was lower in children with good behaviors (126). The reason behind the differences between our results and the two previous studies might be due to choosing N_2O as a sole anesthetic regimen. It has been shown that the cardiovascular and respiratory effects of N_2O can be different from other inhalation agents. For example, while most anesthetic agents increase PR (132), N_2O either does not make a huge impact or decease PR (53,132). Also, unlike other anesthetic agents, N_2O does not decrease net ventilation (132). N_2O has a lower potency and faster onset of action compared with other inhalation agents. It takes N_2O only 30 seconds to impact CNS which is almost equal to lung-brain circulation time, and almost 10 minutes is needed for N_2O to reach the 90% equilibrium (132). Also, as discussed before, the three mechanisms of action of N_2O namely anesthetic, anxiolytic, and analgesic are dose-dependent but independent of each other. Therefore, behavioral and physiological manifestations of N_2O may not be in line with each other or happening at the same time (133).

Besides using the different anesthetic regimens, the other main differences between our study and other reports were the application of audio-visual distraction which has shown to reduce anxiety, promote cooperative behaviors during dental injections, and improve constructive behavioral responses after dental injection. Besides that, it was shown that PR was lower in children undergoing audio-visual distractions (95–97). Also in our study, local anesthesia was administrated using a controlled local anesthetic delivery which decreases the pain and improves cooperative behavior compared with cartridge syringe in children (99,100). Therefore, less excitatory stimuli were perceived by children which probably caused less arousal and valence by ANS (60,63).

4.7. Limitations and Future Directions

This study was conducted within some limitations. *First,* the perception of children which is the third component of emotional response was not measured in this study. Measuring the perception/experience of the children during the dental treatment was not feasible in a real dental setting without changing the environment since the nasal hood was in place during the whole procedure and children could not communicate freely. Asking questions during the dental treatment would disrupt the treatment delivery by the clinicians. Also, asking the children after the dental procedure about their perception would not be reliable as well since it has been shown that experiencing an event is not equal to the memory of the event since narrative and experiencing selves are not the same (58). Besides that, cognitive functions that are impaired during the sedation will not recover up to15 minutes after discontinuation of the N_2O (135). The same delay also has been reported for reaction time after discontinuation of N_2O (136). Therefore, future studies should apply an interventional design to measure the perception of children to provide a better understating of the emotions.

Second, the minimum measuring time for each of the time points in our study was 150 seconds. Although this time duration is enough to observe the gas effect, a longer time duration for the effects to be exposed fully might have been more desirable. Again, extending the minimum duration of each time point would not be feasible unless the real dental settings and environment were changed. Future studies with interventional design instead of observational design can help to better measure the full effect of each condition pertaining to each time point.

Third, when interpreting the results, it should be noted that the audio-visual distraction was used during the dental treatment and local anesthesia was administrated using a controlled local anesthetic delivery system. Since these new modifications to the treatment procedure can impact both behaviors and vital signs and their possible interactions with other factors, the results should be compared with studies that implement the same designs.

Fourth, although not part of our study objectives, we did not follow up on the children after the study for possible side effects of N_2O such as nausea and vomiting related to the treatment procedure. It is suggested that future studies follow up patients up to 24 hours after the dental procedure for any possible side effects.

Fifth, when comparing the results with studies in other medical fields, it should be noted that the delivered concentration of N_2O is different from the concentration reaching alveoli in dental settings. The concentration of nitrous oxide in dental settings is considerably reduced during the delivery process from the machine to the mask, pharynx, and venous blood (132).

Sixth, other important vital signs such as blood pressure and body temperature which can be used as a proxy for ANS response should be measured in future studies. Also, future studies should measure pain during different time points of the dental procedure and investigate its possible role and interaction with the physiological responses and behaviors of children.

5. CONCLUSIONS

Within the limitations of this study, the results suggested that using N_2O can be considered safe and clinically acceptable by observing only minimal fluctuations in the measured vital signs. Improved cooperation and less distress were seen after the administration of N_2O. During the dental procedure, dental injection seemed to lead to the highest fluctuations in vital signs and worst behaviors. The possible effect of N_2O was not dependent on the predictors of the study meaning that N_2O can have an impact on vital signs and behaviors regardless of the participants' demographics, degree of anxiety, parenting style, and treatment duration. Vital signs could not be predicted from behaviors, highlighting that the emotions of children cannot be understood by just observing the behaviors.

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Appendices

Appendix 1. Parenting Style and Dimension Questionnaire (PSDQ-32) (short version)

Parenting Style and Dimension Questionnaire (PSDQ-32)

Make two ratings for each item; (1) rate how often your spouse exhibits this behavior with your child and (2) how often you exhibit this behavior with your child.

Spouse Exhibits Behavior:	**I Exhibit This Behavior:**
I =Never	1 = Never
2 =Once in a While	2 =Once in a While
3 =About Half of the Time	3 =About Half of the Time
4 =Very Often	4 =Very Often
5 =Always	5 =Always

[He/She] [I]

1 _______ ___ [He/She is][I am] responsive to our child's feelings or needs.

2 _______ ___ [He/She uses][I use] physical punishment as a way of disciplining our child.

3 _______ ___[He/She takes][I take] our child's desires into account before asking the child to do something.

4 _______ ___ When our child asks why (he)(she) has to conform, [I state][He/She states]: because I said so or I am your parent and I want you to.

5 _______ ___ [He/She explains][I explain] to our child how we feel about the child's good and bad behavior

6 _______ ___ [He/She spanks][I spank] when our child is disobedient.

7 _______ ___ [He/She encourages] [I encourage] our child to talk about the child's troubles.

8 _______ ___ [He/She finds][I find] it difficult to discipline our child.

9 _______ ___ [He/She encourages][I encourage] our child to freely express (himself)(herself) even when disagreeing with parents

10 _______ ___ [He/She punishes][I punish] by taking privileges away from our child with little if any explanations.

11 _______ ___ [He/She emphasizes] [I emphasize] the reasons for rules.

12 _______ ___ [He/She gives] [I give] comfort and understanding when our child is upset

13 _______ ___ [He/She yells or shouts][I yell or shout] when our child misbehaves.

14 _______ ___ [He/She gives][I give] praise when our child is good.

15 ______ ___ [He/She gives][I give] into our child when the child causes a commotion about something.

16 _______ ___ [He/She explodes][I explode] in anger towards our child.

17_______ ___ [He/She threatens][I threaten] our child with punishment more often than actually giving it.

18 _______ ___ [He/She takes][I take] into account our child's preferences in making plans for the family

19 _______ ___ [He/She grabs] [I grab] our child when he/she is being disobedient

20 _______ ___ [He/She states] [I state] punishments to our child and do not actually do them.

21 _______ ___ [He/She shows][I show] respect for our child's opinions by encouraging our child to express them.

22 _______ ___ [He/She allows][I allow] our child to give input into family rules.

23 _______ ___ [He/She scolds and criticizes] [I scold and criticize] to make our child improve.

24 _______ ___ [He/She spoils][I spoil] our child.

25 _______ ___ [He/She gives][I give] our child reasons why rules should be obeyed.

26 _______ ___ [He/She uses][I use] threats as punishment with little or no justification.

27 _______ ___ [He/She has][I have] warm and intimate times together with our child

28 _______ ___ [He/She punishes][I punish] by putting our child off somewhere alone with little if any explanations

29 _______ ___ [He/She helps][I help] our child to understand the impact of behavior by encouraging our child to talk about the consequences of his/her own actions.

30 _______ ___ [He/She scold][I scold] or criticize when our child's behavior doesn't meet our expectations

31 _______ ___ [He/She explains][I explain] the consequences of the child's behavior.

32 _______ ___ [He/She slaps][I slap] our child when the child misbehaves

Appendix 2. The Spence Children Anxiety Scale-Parent Report

SPENCE CHILDREN'S ANXIETY SCALE
(Parent Report)

Your Name: ____________________ **Date:** ____________________

Your Child's Name: ____________________

BELOW IS A LIST OF ITEMS THAT DESCRIBE CHILDREN. FOR EACH ITEM PLEASE CIRCLE THE RESPONSE THAT BEST DESCRIBES YOUR CHILD. PLEASE ANSWER ALL THE ITEMS.

1.	My child worries about things..	Never	Sometimes	Often	Always
2.	My child is scared of the dark...	Never	Sometimes	Often	Always
3.	When my child has a problem, s(he) complains of having a funny feeling in his / her stomach	Never	Sometimes	Often	Always
4.	My child complains of feeling afraid..	Never	Sometimes	Often	Always
5.	My child would feel afraid of being on his/her own at home................	Never	Sometimes	Often	Always
6.	My child is scared when s(he) has to take a test.................................	Never	Sometimes	Often	Always
7.	My child is afraid when (s)he has to use public toilets or bathrooms.....	Never	Sometimes	Often	Always
8.	My child worries about being away from us / me.................................	Never	Sometimes	Often	Always
9.	My child feels afraid that (s)he will make a fool of him/herself in front of people..	Never	Sometimes	Often	Always
10.	My child worries that (s)he will do badly at school..............................	Never	Sometimes	Often	Always
11.	My child worries that something awful will happen to someone in our family..	Never	Sometimes	Often	Always
12.	My child complains of suddenly feeling as if (s)he can't breathe when there is no reason for this..	Never	Sometimes	Often	Always
13.	My child has to keep checking that (s)he has done things right (like the switch is off, or the door is locked)..	Never	Sometimes	Often	Always
14.	My child is scared if (s)he has to sleep on his/her own........................	Never	Sometimes	Often	Always
15.	My child has trouble going to school in the mornings because (s)he feels nervous or afraid...	Never	Sometimes	Often	Always
16.	My child is scared of dogs	Never	Sometimes	Often	Always
17.	My child can't seem to get bad or silly thoughts out of his / her head........	Never	Sometimes	Often	Always
18.	When my child has a problem, s(he) complains of his/her heart beating really fast..	Never	Sometimes	Often	Always

19.	My child suddenly starts to tremble or shake when there is no reason for this..........	Never	Sometimes	Often	Always
20.	My child worries that something bad will happen to him/her..........	Never	Sometimes	Often	Always
21.	My child is scared of going to the doctor or dentist	Never	Sometimes	Often	Always
22.	When my child has a problem, (s)he feels shaky..........	Never	Sometimes	Often	Always
23.	My child is scared of heights (eg. being at the top of a cliff)..........	Never	Sometimes	Often	Always
24.	My child has to think special thoughts (like numbers or words) to stop bad things from happening..........	Never	Sometimes	Often	Always
25.	My child feels scared if (s)he has to travel in the car, or on a bus or train	Never	Sometimes	Often	Always
26.	My child worries what other people think of him/her..........	Never	Sometimes	Often	Always
27.	My child is afraid of being in crowded places (like shopping centres, the movies, buses, busy playgrounds)..........	Never	Sometimes	Often	Always
28	All of a sudden my child feels really scared for no reason at all..........	Never	Sometimes	Often	Always
29.	My child is scared of insects or spiders..........	Never	Sometimes	Often	Always
30.	My child complains of suddenly becoming dizzy or faint when there is no reason for this..........	Never	Sometimes	Often	Always
31.	My child feels afraid when (s)he has to talk in front of the class..........	Never	Sometimes	Often	Always
32.	My child's complains of his / her heart suddenly starting to beat too quickly for no reason	Never	Sometimes	Often	Always
33.	My child worries that (s)he will suddenly get a scared feeling when there is nothing to be afraid of..........	Never	Sometimes	Often	Always
34.	My child is afraid of being in small closed places, like tunnels or small rooms..........	Never	Sometimes	Often	Always
35.	My child has to do some things over and over again (like washing his / her hands, cleaning or putting things in a certain order)..........	Never	Sometimes	Often	Always
36.	My child gets bothered by bad or silly thoughts or pictures in his/her head	Never	Sometimes	Often	Always
37.	My child has to do certain things in just the right way to stop bad things from happening	Never	Sometimes	Often	Always
38.	My child would feel scared if (s)he had to stay away from home overnight..........	Never	Sometimes	Often	Always
39.	Is there anything else that your child is really afraid of?	YES	NO		
	Please write down what it is, and fill out how often (s)he is afraid of this thing: ____________________	Never	Sometimes	Often	Always
	____________________	Never	Sometimes	Often	Always
	____________________	Never	Sometimes	Often	Always

Appendix 3. The Preschool Anxiety Scale-Parent Report

PRESCHOOL ANXIETY SCALE
(Parent Report)

Your Name: ____________________ **Date:** ____________________

Your Child's Name: ____________________

Below is a list of items that describe children. For each item please circle the response that best describes your child. Please circle the **4** if the item is **very often true, 3** if the item is **quite often true, 2** if the item is **sometimes true, 1** if the item is **seldom true** or if it is **not true at all** circle the **0**. Please answer all the items as well as you can, even if some do not seem to apply to your child.

		Not True at All	Seldom True	Sometimes True	Quite Often True	Very Often True
1	Has difficulty stopping him/herself from worrying…………………	0	1	2	3	4
2	Worries that he/she will do something to look stupid in front of other people……………………………………………………….….	0	1	2	3	4
3	Keeps checking that he/she has done things right (e.g., that he/she closed a door, turned off a tap)………………..	0	1	2	3	4
4	Is tense, restless or irritable due to worrying………………………	0	1	2	3	4
5	Is scared to ask an adult for help (e.g., a preschool or school teacher)………………………………………………………………..	0	1	2	3	4
6	Is reluctant to go to sleep without you or to sleep away from home……………………………………………………………….	0	1	2	3	4
7	Is scared of heights (high places)…………………………………….	0	1	2	3	4
8	Has trouble sleeping due to worrying………………………………	0	1	2	3	4
9	Washes his/her hands over and over many times each day…….	0	1	2	3	4
10	Is afraid of crowded or closed-in places……………………………..	0	1	2	3	4
11	Is afraid of meeting or talking to unfamiliar people…………………	0	1	2	3	4
12	Worries that something bad will happen to his/her parents………..	0	1	2	3	4
13	Is scared of thunder storms…………………………………………..	0	1	2	3	4
14	Spends a large part of each day worrying about various things.….	0	1	2	3	4
15	Is afraid of talking in front of the class (preschool group) e.g., show and tell…………………………….…………………….	0	1	2	3	4
16	Worries that something bad might happen to him/her (e.g., getting lost or kidnapped), so he/she won't be able to see you again………………………………………………………………..	0	1	2	3	4
17	Is nervous of going swimming………………………………………...	0	1	2	3	4

		Not True at All	Seldom True	Sometimes True	Quite Often True	Very Often True
18	Has to have things in exactly the right order or position to stop bad things from happening…………………………………………	0	1	2	3	4
19	Worries that he/she will do something embarrassing in front of other people……………………………………………………………	0	1	2	3	4
20	Is afraid of insects and/or spiders……………………………………	0	1	2	3	4
21	Has bad or silly thoughts or images that keep coming back over and over…………………………………………………………………	0	1	2	3	4
22	Becomes distressed about your leaving him/her at preschool/school or with a babysitter………………………………..	0	1	2	3	4
23	Is afraid to go up to group of children and join their activities……..	0	1	2	3	4
24	Is frightened of dogs……………………………………………………	0	1	2	3	4
25	Has nightmares about being apart from you………………………..	0	1	2	3	4
26	Is afraid of the dark……………………………………………………	0	1	2	3	4
27	Has to keep thinking special thoughts (e.g., numbers or words) to stop bad things from happening………………………………………	0	1	2	3	4
28	Asks for reassurance when it doesn't seem necessary……………	0	1	2	3	4
29	Has **your child ever experienced anything really bad or traumatic** (e.g., severe accident, death of a family member/friend, assault, robbery, disaster) …………………………	**YES**	**NO**			

Please briefly describe the event that your child experienced……

____

____

____

____

____

If you answered **NO** to **question 29,** please **do not** answer questions 30-34**. If you answered YES, please DO** answer the following questions**.**

Do the following statements describe your child's behaviour since the event?

		Not True at All	Seldom True	Sometimes True	Quite Often True	Very Often True
30	Has bad dreams or nightmares about the event……………………	0	1	2	3	4
31	Remembers the event and becomes distressed……………………	0	1	2	3	4
32	Becomes distressed when reminded of the event…………………	0	1	2	3	4
33	Suddenly behaves as if he/she is reliving the bad experience……	0	1	2	3	4
34	Shows bodily signs of fear (e.g., sweating, shaking or racing heart) when reminded of the event …………………………………	0	1	2	3	4

Appendix 4. Frankl Scale

Ratings	**Description**
F1: Definitely Negative	Refusal of treatment, crying forcefully, fearful, or any other overt evidence of extreme negativism.
F2: Negative	Reluctant to accept treatment, uncooperative, some evidence of negative attitude but not pronounced i.e. sullen, withdrawn.
F3: Positive	Acceptance of treatment; at times caution. Willingness to comply with the dentist, at times with reservation but the patient follows the dentist's direction cooperatively.
F4: Definitely Positive	Good rapport with the dentist, interested in the dental procedure, laughing and enjoying the situation