The Impact of a Humanoid Robot on Children’s Dental Anxiety, Behavior and Salivary Amylase Levels: A Randomized Clinical Trial

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ABSTRACT
Aim: With the spread of technological possibilities, the idea that humanoid robots can accompany medical interventions has gained momentum. The objectives of this two-armed randomized controlled study were (i) to assess the effects of a human-like robot on behavior guidance during children’s dental treatment, by a comparison of the dental anxiety scale, behavioral scale, pulse rate and amylase levels in the saliva and (ii) to determine whether the children would like to have treatment with a humanoid robot.

Materials and Methods: One hundred two children (52 girls, 50 boys; mean age: 6.71±1.43 years) were included. The exclusion criteria were children showing definitively negative behavior (Frankl 1) during dental prophylaxis at the first visit and those children who had had dental treatment previously. Fifty children were assigned to the robot group (RG) and 52 children were assigned to the control group (CG). The Facial Image scale (FIS), Frankl Behaviour Rating scale (FBRS), physiological pulse rate and salivary alpha amylase (sAA) levels were used to assess the stress related changes. The Mann-Whitney U test and the Student’s t-test were used to compare the groups. In-group comparisons were tested with the Wilcoxon signed-rank test. The chi-squared test, Continuity (Yates) correction, and the Fisher-Freeman-Halton test were used to compare qualitative data. Statistical tests were considered significant at a p-value set at 0.05.

Results: The post-treatment FIS scores of the RG in the 6-10-year-olds were significantly lower than in the CG (p<0.05). Post-treatment FBRS scores were statistically significantly higher in the RG than in the CG in both younger and older children (p<0.05). In children aged between 6 and 10 years, pulse rates during and after treatment in the RG were significantly lower than those in the CG (p<0.05). No correlation was found between the children’s anxiety/behavior and their sAA levels.

Conclusion: The robot was found to be effective in reducing dental anxiety and pulse rates in children aged 6-10 years, and it was preferred more by the children of this age.

Keywords: Dental anxiety, human computer interaction, robotic, salivary amylase

Introduction
Despite modern technological advances, dental procedures still cause anxiety and fear reactions. Dental anxiety is a very common condition in children who receive dental treatment and it can cause problems for the dentist and the patient alike (1). Although dental anxiety can be seen at any age, it usually occurs in childhood. The dental anxiety problem seen in childhood may continue...
into adulthood, causing people to avoid dental treatment and consequently negatively affect their oral and dental health (2).

Various psychological and pharmacological techniques are used to insulate pediatric patients from dental anxiety during their dental treatment. Behavior guidance techniques are based on understanding the social, emotional and cognitive development of children in order to provide effective treatment and establish social behavioral guidance. Non-verbal communication, distraction, positive encouragement, voice control, and tell-show-do are among the non-pharmacological behavioral guidance techniques (3).

We are in an era of the increasing development and testing of social robots in the medical field (4). There are over a dozen existing applications with healthcare robots including changing dressings, blood tests, catheter insertion/removal, oxygen tube insertion, IV start/removal, vaccinations, and electroencephalogram (5,6). There are robots with different applications which attract the attention of children, train them and support them with cognitive-behavioral interventions e.g. managing illnesses by promoting the correct behavior in those children with chronic health conditions, helping to distract children undergoing acute medical procedures or during vaccinations or comforting them as a friend (7).

There is an increasing focus on children's interactions with robots. Socially assistive robots have the ability to assist to ease the procedures in order to reduce children's anxiety and distress during their hospital visits (8-10). NAO is a programmable, autonomous humanoid robot. The NAO has been used to develop adaptive behaviors in children in a number of studies (11-14). However, there was no study to-date about the use of social robots in dentistry for reducing dental fear and anxiety.

Saliva can be analyzed for biomarkers as it reflects many systemic and local biochemical and physiological processes. The non-invasive and easy nature of saliva sampling make it a very useful and relatively stress-free diagnostic alternative to blood sampling in neuroscience and psychology. Therefore, it is of value in studying anxiety towards a dental procedure. Saliva secretion is regulated by the autonomic nervous system (ANS). sAA has been suggested as an index of autonomic activity as a result of its release from the salivary glands being under the strong control of the local sympathetic nerves and so its salivary concentration rapidly increases in certain circumstances (15,16). As an ANS marker, sAA is a valid indicator for acute sympathetic nervous system stress response (16). sAA is more sensitive to subtle psychological stress compared to systolic blood pressure or pulse rates (17).

The primary objective of this study was to assess the effects of a human-like robot on the behavior guidance during children's dental treatment, by comparisons of the dental anxiety scale, the behavioral scale, pulse rates and amylase levels in the saliva. Our secondary objective was to determine whether the children in the RG would like to have future treatment with the NAO.

**Materials and Methods**

This randomized controlled clinical trial was approved by the Istanbul University Faculty of Dentistry Clinical Research Ethics Committee (2014/461) and it was conducted within ethical standards in accordance with the Declaration of Helsinki. Informed consent was obtained from all of the children and their parents. This study was carried out according to the CONSORT 2010 statement (18). The study protocol was registered on ClinicalTrials (NCT05238246).

The following focused question was developed in accordance with the recognized Patient, Intervention, Comparison, and Outcome (PICO) method: In children who were between 4 and 10 years old, during their first experience with the dentist and in need of restoration or a pulpotomy (P); what was the effect of techno-psychological distraction (I) as compared to the conventional behavior management method (C)? Was there any decrease in dental anxiety observed by physiological or psychological measurements (O)?

**Selection and Description of Participants**

Healthy children aged between 4 and 10 years, having their first dental visit and requiring a pulpotomy for a primary molar were selected. We included only children aged 4 years and older in our study, as younger infants have low cooperation skills. Those children who agreed to complete the questionnaires and who gave informed consent and were accompanied by at least one parent participated in this study. Children who had a history of systemic diseases or who were medically compromised were not included in this study.

Inclusion criteria were (a) healthy children who did not have any genetic syndrome or systemic diseases in their medical history, who were not physically or mentally disabled, (b) those who had had no previous dental experience, (c) and who needed pulpotomy in at least one mandibular primary molar.
Those children with a significant systemic disease in their medical history or with disabilities, and those who had previously been taken to the dentist for treatment, and those who were unaccompanied or who refused to fill out the questionnaire, those children who exhibited definitely negative behavior (Frankl I) during their dental prophylaxis at the first visit and those children who had had dental treatment before were not included in this study.

The G*Power program was used to determine the number of participants, the effect size for the facial image scale (FIS) score (19) was \( d \) (effect size): 0.637, SD: 1.25, Power: 0.80 and \( \alpha \): 0.05, and the minimum number of participants for each group was calculated to be \( n=22 \).

**Sample Characteristics**

One hundred and two children who met the inclusion criteria took part in this research. Fifty children were enrolled in the robot group (RG) and fifty-two children were enrolled in the control group (CG). The mean age of all of the children was 6.71±1.43 years (Table I). The CONSORT flow chart for participant enrollment is shown in Figure 1.

### Randomization

Participants were randomized in a 1:1 ratio for each age (year) and gender to the groups. To identify the order of intervention in each treatment group, block randomization was used in this study. A table of random numbers was used to generate the random allocation sequence. One pediatric dentistry resident (S.K.) enrolled participants and one pediatric dentist (E.B.T.) assigned the participants to the interventions. A pediatric dentist (Y.K.) found out about the patient’s group just before the treatment session. Due to

| Table I. Gender and age distribution of the participants |
|-----------------|--------|--------|--------|
| Gender          | RG     | %      | CG     | %      |
| Male            | 24     | 48     | 26     | 50     |
| Female          | 26     | 52     | 26     | 50     |
| Age (years)     |        |        |        |        |
| 4-5             | 8      | 16     | 14     | 26.9   |
| 6-10            | 42     | 84     | 38     | 73.1   |

RG: Robot group, CG: Control group

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Figure 1. CONSORT flow chart
to the nature of the treatments provided, blinding for FBRS assessment was not possible.

**Treatment Procedure**

The study protocol was explained to the parents and their written consent was obtained. Pre-treatment questionnaires were asked to the participants.

All children were treated under inferior alveolar block anesthesia. The treatments were performed by the same pediatric dentist (Y.K.). The RG were treated with the robot accompaniment. The CG were treated without the robot. The treatments were completed in the same session.

In the robot group, the distraction technique were achieved by means of the humanoid robot. The robot used in experiments was the NAO, manufactured by Softbank Robotics. NAO is a 58-cm tall robot which is able to perform targeted motor tasks. The robot was programmed to perform the same series of instructions for every patient in order to guarantee that all of the children had the same experience with the robot. The movements of the robot were controlled wirelessly from a computer by using Choregraphe (Aldebaran Robotics, France) software. We prepared the transactions to let the operator run the corresponding commands for each period of the treatment in real time in order to create sequences of behaviors. After each task was defined individually, it was assigned a keyboard input. Some combinations of robot movements were achieved by pressing a single key. With this keyboard interface, the learning time of the operator was minimized. During this period, the system to control the robot was simplified and mistakes were reduced. The NAO audibly and visually distracted the children (Figure 2).

After the dental intervention, the children who were in the robot group were asked “Would you like to see the NAO again in your next appointment?”. 

**Anxiety Status**

A series of questionnaires measuring anxiety were administered to each child, before and after their treatment.

FIS is a commonly used scale to determine the dental anxiety levels of children. The children were asked to point at FIS before treatment. This scale consists of five faces numbered from 1 to 5 and ranging from “very happy” to “very unhappy”. Each child was asked to point to the face that they related to most closely, according to their feelings at that moment. The questionnaire was repeated after their treatment. FIS is a validated tool for children aged 3-18 years to express their dental anxiety (19).

**Behavior Assessment**

Frankl’s behavior rating scale (FBRS) was used by the dentist to assess each child’s behavior (20). It is considered to be one of the most reliable tools developed for the behavior rating of children in dental settings (21). The child's

![Figure 2. Operation of the robot](image-url)
behavior is classified by one of the following: definitely negative, negative, positive or definitely positive. The child’s behavior was evaluated at their first visit. Children with FBRS scores of 1 were excluded from this study. FBRS was repeated at the end of the treatment sessions.

**Physiologic Monitoring**

**Pulse rate**

Monitoring the pulse rate allows for real-time and continuous measurements at different phases of the dental treatment (22). Pulse oximeters were used to measure physiological pulse rates (bpm) before, during and after the treatment.

**Salivary amylase activity**

Salivary alpha amylase can be used as a reliable objective tool to measure anxiety during dental treatment (23). Saliva was collected using the “spitting method” (24). Each subject rinsed their mouth with water to reduce contamination of saliva with food debris and waited 5 minutes prior to sampling. Whole mouth saliva from the oral cavity was collected by asking the subjects to sit comfortably in an upright position and drop down their heads, let the saliva run naturally to the front of mouth without stimulating flow by means of orofacial movements. The saliva which accumulated in the floor of the mouth was expectorated into a graduated polypropylene test tube every 30 seconds for a total of 2 minutes. The amount of collected saliva in mL divided by the duration of the collection period, yielding ml/min, was recorded as the mean salivary flow rate.

The saliva samples were collected at three time points as follows: the first saliva samples of the children were collected 5 minutes before the dental treatment (pre-5, measuring the stress of being at the clinic). The patients then underwent dental procedures which lasted around 30 minutes. Right after dental treatment and after 10 minutes of resting (post-10), two new saliva samples were collected (Figure 1). Collection of at least 1 mL was required. After collection, the unprocessed samples were stored at -20 °C until they were analyzed.

Salivary alpha-amylase was measured by a colorimetric assay using 4,6-ethylidene-(G7)p-nitrophenyl-(G1)-D-maltoheptaoside (ethylidene-G7PNP) in an automatic analyzer (Cobas Integra 800, Roche, Basel, Switzerland). The diluted (1:400) saliva samples were assayed.

**Children’s attitudes towards the robot**

After the dental intervention, the children who participated in RG were asked “Would you like to see the NAO again at your next appointment?” Their responses were rated as a minimum of 1 and a maximum of 5.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all</td>
<td>Not really</td>
<td>Undecided</td>
<td>Somewhat</td>
<td>Very much</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

IBM SPSS Statistics 22 (IBM SPSS, Turkey) was used for the statistical analysis. The Shapiro-Wilk test and the Kolmogorov-Smirnov test were used to check the data distribution. The Mann-Whitney U test and the Student’s t-test were used to compare two groups. In-group comparisons of non-normally distributed parameters were tested with the Wilcoxon signed rank test. The chi-squared test, continuity (Yates) correction, and the Fisher-Freeman-Halton test were used to compare qualitative data. Pearson correlation analysis was used to examine the correlation between parameters for normal distribution, and Spearman’s rho correlation analysis was used to examine correlations between those parameters with normal distribution. Statistical significance was determined as p<0.05. The various age ranges contain children in different development stages, so this variable might have influenced the results. Therefore, we divided the children into two subgroups, namely “preschool” and “school term” children.

**Results**

**Anxiety status**

The Mann-Whitney U test was used for FIS comparison between the robot and the control groups, and the Wilcoxon sign test was used for intra-group comparisons before and after treatment.

There was no statistically significant difference between the groups in terms of their mean FIS scores before and after treatment in the 4-5-year-old children. However, the post-treatment FIS scores of the robot group in the 6-10-year-old children was statistically significantly lower than in the control group (p<0.05) (Table II).

**Behavior assessment**

The Mann-Whitney U test was used for FBRS comparison between the robot and the control groups, and the Wilcoxon sign test was used for intra-group comparisons before and
after treatment. The behavior of the children was assessed by one pediatric dentist (EBT) (intra-class correlation coefficient score=0.87). The post-treatment FQRS score was statistically significantly higher in the robot group than in the control group (p<0.05) (Table II).

**Pulse rate**

Student’s t-test was used for pulse rate comparisons between the robot and the control groups, analysis of variance was used in repeated measurements before, during and after treatment for the groups, and the Bonferroni test was used for pairwise comparisons within the groups.

There was no significant difference between the robot group and the control group in the 4-5-year-old children. However, for children aged 6-10 years, pulse rates during and after treatment in the robot group were significantly lower than for those in the control group (p<0.05) (Table III).

**Salivary amylase activity**

Student’s t-test was used for sAA comparison between the robot and the control groups, analysis of variance in repeated measurements was used for in-group comparisons, and the Bonferroni test was used for pairwise comparisons within groups.

There was no statistically significant difference between the groups in terms of the pre-1, post-1 and post-10 sAA levels in all children. The highest sAA values were observed at the end of treatment in both groups. However, a significant difference was found in the 4-5-year-old children in the control group (p<0.05). When comparing the post-treatment values, there were marked decreases in the sAA which were seen to fall to below pre-treatment levels in the robot group (p<0.05) (Figures 3 and 4) (Table IV).

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**Table II. Comparison of the participants’ anxiety and behavior before and after treatment**

<table>
<thead>
<tr>
<th></th>
<th>4-5-year-olds</th>
<th>6-10-year-olds</th>
<th>1 ^p-value</th>
<th>2 ^p-value</th>
<th>1 ^p-value</th>
<th>2 ^p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RG</td>
<td>CG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FIS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.43±1.51 (2)</td>
<td>2.36±1.69 (1.5)</td>
<td>0.694</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>2.29±1.38 (2)</td>
<td>2.93±1.59 (2.5)</td>
<td>0.396</td>
<td>1.31±0.68 (1)</td>
<td>1.84±1.2 (1)</td>
<td>0.019*</td>
</tr>
<tr>
<td>^p-value</td>
<td>0.705</td>
<td>0.291</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FBRS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.57±0.53 (3)</td>
<td>2±0 (2)</td>
<td>0.002*</td>
<td>2.63±0.58 (3)</td>
<td>2.50±0.56 (2)</td>
<td>0.215</td>
</tr>
<tr>
<td>After</td>
<td>3±1 (3)</td>
<td>2.14±0.86 (2)</td>
<td>0.046*</td>
<td>3.52±0.63 (4)</td>
<td>2.74±0.72 (3)</td>
<td>0.000*</td>
</tr>
<tr>
<td>^p-value</td>
<td>0.180</td>
<td>0.527</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Mann-Whitney U Test, 2 Wilcoxon sign test, *p<0.05

**RG: Robot group, CG: Control group, FIS: Facial image scale, FBRS: Frankl’s behavior rating scale, SD: Standard deviation**

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**Table III. Comparison of the participants’ pulse rates before, during and after treatment**

<table>
<thead>
<tr>
<th></th>
<th>4-5-year-old</th>
<th>6-10-year-old</th>
<th>1 ^p-value</th>
<th>2 ^p-value</th>
<th>3 ^p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RG</td>
<td>CG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pulse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>110.50±14.71</td>
<td>108.0±12.90</td>
<td>0.682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During treatment</td>
<td>120.29±25.70</td>
<td>113.64±13.38</td>
<td>0.440</td>
<td>103.45±16.05</td>
<td>110.92±15.86</td>
</tr>
<tr>
<td>After treatment</td>
<td>103.86±18.06</td>
<td>104.0±11.24</td>
<td>0.982</td>
<td>95.93±11.90</td>
<td>107.08±13.81</td>
</tr>
<tr>
<td>^p-value</td>
<td>0.013*</td>
<td>0.038*</td>
<td></td>
<td>0.002*</td>
<td>0.024*</td>
</tr>
<tr>
<td>Before/After ^p-value</td>
<td>0.463</td>
<td>0.855</td>
<td></td>
<td>1.000</td>
<td>0.018*</td>
</tr>
<tr>
<td>Before/Rest ^p-value</td>
<td>0.947</td>
<td>0.986</td>
<td></td>
<td>0.003*</td>
<td>0.261</td>
</tr>
<tr>
<td>After/Rest ^p-value</td>
<td>0.008*</td>
<td>0.036*</td>
<td></td>
<td>0.009*</td>
<td>0.560</td>
</tr>
</tbody>
</table>

1 Student’s t-test, 2 Analysis of variance in repeated measurement, 3 Bonferroni test, *p<0.05

**RG: Robot group, CG: Control group, SD: Standard deviation**
Figure 3. Change of sAA level at three-time measures of children (age 4-5) before and after dental treatment
RG: Robot group, Pre-5: 5 min before the dental treatment, Post-1: immediately after dental treatment, Post-10: 10 min after dental treatment.

Figure 4. Change of sAA level at three-time measures of children (age 6-10) before and after dental treatment
RG: Robot group, Pre-5: 5 min before the dental treatment, Post-1: immediately after dental treatment, Post-10: 10 min after dental treatment

Table IV. Changes in sAA values in children aged 4-5 and 6-10 years old who underwent dental treatment

<table>
<thead>
<tr>
<th></th>
<th>4-5 year-old</th>
<th>6-10 year-old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RG</td>
<td>CG</td>
</tr>
<tr>
<td>sAA (U/mL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Pre-5</td>
<td>233.79±69.55</td>
<td>176.96±52.45</td>
</tr>
<tr>
<td>Post-1</td>
<td>255.08±62.56</td>
<td>217.9±74.13</td>
</tr>
<tr>
<td>Post-10</td>
<td>229.01±59.91</td>
<td>209.48±69.27</td>
</tr>
<tr>
<td>'p-value'</td>
<td>0.002*</td>
<td>0.018*</td>
</tr>
<tr>
<td>Pre-5/Post-1 'p-value'</td>
<td>0.085</td>
<td>0.035*</td>
</tr>
<tr>
<td>Pre-5/Post-10 'p-value'</td>
<td>1.000</td>
<td>0.023*</td>
</tr>
<tr>
<td>Post-1/Post-10 'p-value'</td>
<td>0.048*</td>
<td>1.000</td>
</tr>
</tbody>
</table>

'p-value': Student’s t-test, Analysis of variance in repeated measurements, Bonferroni Test, *p<0.05
SD: Standard deviation, RG: Robot group, CG: Control group, Pre-5: 5 mins before the dental treatment, Post-1: immediately after dental treatment, Post-10: 10 mins after dental treatment

Table V. Comparison of the children's willingness to encounter NAO at their next appointment

<table>
<thead>
<tr>
<th></th>
<th>Willing to have the NAO in the next appointment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>4.79±0.66</td>
</tr>
<tr>
<td>Girls</td>
<td>4.65±0.56</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>4-5</td>
<td>4.0±1.07</td>
</tr>
<tr>
<td>6-10</td>
<td>4.86±0.35</td>
</tr>
</tbody>
</table>

Mann-Whitney U test, *p<0.05
SD: Standard deviation
Children's attitudes towards the robot

The Mann-Whitney U test was used for comparisons between the genders and age groups to ascertain any differences in the children's eagerness to see the robot (NOA) again.

There was no statistically significant difference between the girls and the boys in terms of wanting the robot accompaniment in their appointments. The 6-10-year-old children were more likely to want to be accompanied by the robot in their appointments than the 4-5-year-olds (p<0.05) (Table V).

Discussion

Humanoid robots have been increasingly used in the healthcare system to provide cognitive-behavioral support to patients. In recent years, research on humanoid robots helping to care for the elderly has intensified. Robots may help in the education of children with chronic health problems or special needs such as autism, in the development children's skills, in encouraging children to acquire healthy behaviors, and in making children comfort. However, there are few studies on humanoid robots helping children in the medical field.

The NAO robot is a humanoid robot which can be used in education and therapy. The NAO robot can be used as an educator and as well as a peer of children. It has been used in the classroom to teach new words to children between 3 and 6 years old (25), to assist in speech therapy (12), to adapt children to learn a second language in kindergartens (26), to improve the efficacy of nutritional education (27), and to deliver motivational interviews (28). The NAO robot has mostly been used to improve social behavior and to improve the quality of life in children with autism to date (11,26), and to screen for autism in toddlers, as reported in the literature (29). As NAO has a positive interaction with children, we decided to have NAO to guide, distract and encourage children during their dental treatment.

The distraction method is used to shift the perception of pain to an alternative stimulus. Various tools are used for distraction, from simple interventions to advanced methods such as virtual reality. Two studies were found in which humanoid robots were used as a distraction tool in medical procedures. Beran et al. (30) programmed a robot to distract children during vaccinations and Ali et al. (31) used robot-based distraction therapy in children undergoing intravenous insertion. However, studies on the use of robots to reduce dental anxiety have not yet become widespread.

Virtual reality, one of the most recently developed techniques, is increasingly used in pediatric dentistry to reduce anxiety and pain during local anesthesia or painful procedures such as extraction (32-34). Although no other study has been conducted on the use of humanoid robots in pediatric dentistry apart from ours, humanoid robots, which are complex technology products, can affect pain perception and dental anxiety more effectively than virtual reality because they can be both visually and audibly distracting and socially interactive.

In this study, it was observed that 6-10-year-old children who had dental treatment with the NAO felt happier and had lower pulse rates than the CG children. The children in the RG exhibited more adaptive behaviors than the CG in both age groups. However, in our opinion, better results could be obtained in younger children by performing the dental treatment after a familiarization session with the NAO.

In research examining the biological basis of behavior, salivary measures have emerged that are minimally invasive, easy-to-collect, and relatively inexpensive markers of stress. In our study, saliva was collected using the “spitting method” under supervision. This method is generally accepted as the preferred method in saliva research and it can be used when the flow rate is low; however, it might have some stimulatory effects (35). Unstimulated whole saliva can be sampled by placing absorbent materials such as a cotton sponge and it is one of the few methods for saliva sampling which is easy to perform even at the home. However, swallowing must be avoided and stimulation of salivary flow cannot be completely excluded. The major disadvantage of absorbent materials is the retention of salivary analytes, including sAA, which introduces measurement error (36). Thus, the spitting method seems appropriate enough to be performed by participants during most experimental setups undertaken in the field of psychoneuroendocrinology (37).

The changes in sAA levels are remarkable given that the same mental stress event or age has no impact on sAA levels (16). The parasympathetic nervous system is inhibited when stressed and the sympathetic nervous system is activated, resulting in decreased saliva production and decreased salivary volume. Uncertainties remain about the possible confounding role of salivary flow rate in determining sAA levels. Some reports indicated that stress-induced increases in amylase activities were not correlated with flow rate (16,38). Other studies suggest that valid measurements of sAA require adjustment for the salivary flow rate (15,39). In this study, there were no significant associations observed with sAA levels.
The most powerful aspect of this study is that it was one of the pioneering studies in dentistry in terms of its novel use of social robots in the behavioral management of children. The NAO was well accepted and appreciated by most of the children. At the same time, the possibility of some preschool children not appreciating the NAO should be considered. Although it attracted the attention of most of the children, its high cost and the need for someone knowledge regarding its technology limit the effectiveness of the NAO. One of the potential biases of this study was that behavioral scoring could not be blinded due to the inability to hide the NAO in the RG. Another of the limitations of this study was the inability to obtain sufficient saliva samples for sAA analysis, especially from those children younger than 6 years of age.

**Conclusion**

As a result of this study, the interaction of children with the NAO during dental treatment was found to be effective in their behavior management. Positive results with the NAO were determined by subjective scales and pulse rate measurements, but no significant difference was found in the sAA levels between the robot and control groups. In the future, robots will be more involved in our daily life and they will be able to accompany and comfort children during their dental treatments.

**Ethics**

**Ethics Committee Approval**: This randomized controlled clinical trial was approved by the Istanbul University Faculty of Dentistry Clinical Research Ethics Committee (2017/6) and it was conducted within ethical standards in accordance with the Declaration of Helsinki.

**Informed Consent**: Informed consent was obtained from all of the children and their parents.

**Peer-review**: Externally and internally peer-reviewed.

**Authorship Contributions**


**Conflict of Interest**: No potential conflict of interest was reported by the authors.

**Financial Disclosure**: The Scientific and Technological Research Council of Türkiye (TÜBİTAK) was supported this study under grant no: 214S157.

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