Salivary Cortisol and Sustained Auditory Attention in Children with and without Cleft Lip and Palate

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Int Arch Otorhinolaryngol 2022;26(4):e605–e614.

Keywords
► hydrocortisone
► child
► attention
► cleft lip
► cleft palate
► cognitive hearing

Abstract

Introduction Cortisol is a hormone involved in the response to stress. Attention is a function that can change due to exposure to stress.

Objectives To verify the correlation between the level of salivary cortisol and sustained auditory attention in children with cleft lip and palate, as well as to compare the results of the variables analyzed between female and male patients.

Methods In total, 103 children aged 6 to 11 years, were divided into 2 groups: those with cleft lip and palate (experimental group, EG; n = 69) and the control group (CG; n = 34). The Sustained Auditory Attention Ability Test (SAAAT) and salivary cortisol levels, measured by an enzyme immunoassay kit (Salimetrics, Stage College, PA, US), were calculated and compared regarding the two groups. The statistical tests used were the Mann-Whitney test and the Spearman correlation.

Results The median level of salivary cortisol was of 0.03615 µl/dL and 0.18000 µl/dL respectively for the EG and CG, with a significant difference between the groups (p = 0.000). Absence of statistical significance (total error score = 0.656; vigilance decrement = 0.051) was observed for the SAAAT among the EG (median total error score = 12.00; 25th percentile [25%] = 7.00; 75th percentile [75%] = 21.00; and vigilance decrement = 1.00; 25% = 0.00; 75% = 2.50) and the CG (median total error score = 12.00; 25% = 6.00; 75% = 24.00; and vigilance decrement = 0.00; 25% = -1.00; 75% = 2.00).

Conclusion All children had median levels of salivary cortisol and scores for sustained auditory attention within normal parameters. A significant correlation between the level of salivary cortisol and the ability to sustain auditory attention was observed in children without cleft lip and palate. There were no differences regarding the SAAAT and salivary cortisol between female and male patients.
Introduction

A highly prevalent craniofacial anomaly worldwide (≈ 1/700 live births), cleft lip and palate (CLP) occurs between the 4th and 12th weeks of intrauterine life, when the embryonic development of the face and palate are taking place. Its etiology is multifactorial, including genetic and environmental factors.\(^1\) In general, the treatment is initiated early, and it involves several specialties. The constant hospitalizations may cause stress, fear, anxiety, morbid fantasies, and elicit feelings of helplessness and weakness from the patients and their families.\(^2\)

Cleft lip and palate are birth defects that affect different structures and functions; they can cause difficulties regarding speech, as well as issues pertaining to esthetics, eating, nutrition etc.,\(^3\) which lead to others that are also important in the lives of people with malformations, and can have significant repercussions in the process of learning, development and understanding of language. Deficits in the sustained auditory attention have been reported by researchers\(^4,5\) in children with CLP. Studies\(^6,7\) have also shown attentional impairments below what is expected for the development stage of children with CLP.

Sustained attention is the ability to concentrate on a specific stimulus over a given period of time,\(^8,9\) while excluding distracting stimuli.\(^8\) Impairments in sustained attention, therefore, may impact the child’s ability to acquire and integrate new skills and knowledge.\(^10\) A previous study\(^11\) suggests that most auditory processing disorders children are not of broader attentional problems, but instead face major deficits in auditory attention tasks (sustained and auditory).

The ability to sustain attention can also be affected by exposure to stressful situations. Researchers\(^12\) have investigated the effects related to time on sustained attention and emotional processing after exposure to a situation of acute stress, and they found a decrease in the level of attention. This was observed mainly in the experimental group (EG), while cortisol was at higher levels than those observed in the control group (CG). The effects of stress on attention skills,\(^13,14\) as well as on cortisol, have also been studied.\(^15,16\)

Cortisol is a corticosteroid that is distributed to all body fluids, such as urine, sweat, blood or saliva. Saliva is a valuable medium due to its easy collection and the possibility of simultaneous evaluation of multiple salivary analytes.\(^17\) Thus, saliva collection for the analysis of the level of cortisol is simple, safe, and non-invasive, and can be performed wherever the participant is.\(^18\) However, to reduce the impact of the diurnal variation in cortisol levels, it is recommended that surveys with saliva collection be performed in the afternoon, between 12 p.m. and 18 p.m., when cortisol levels are relatively stable.\(^15\) In children, researchers have frequently used salivary cortisol because its collection is painless and it is easy to store.\(^19\) Salivary cortisol is a marker of activation of the hypothalamic-pituitary-adrenal (HPA) axis,\(^17\) a neuroendocrine system that regulates the adrenocortical secretion of glucocorticoid steroids to the brain,\(^20\) and is one of the many endogenous systems reactive to stress.\(^21\)

Knowing that patients with CLP need to be hospitalized and spend long periods away from home, that the requirements of the corrective processes can generate great stress for the patient, especially in early childhood, when the fear of the unknown is a very striking factor, that cortisol levels can be elevated by stress, and that attention can change under stress, the present research aims to verify the correlation between the level of salivary cortisol and sustained auditory attention in children with CLP, as well as to compare the results of the variables analyzed between female and male patients.

Methods

The present was an observational, analytical, cross-sectional and quantitative study conducted after approval from the Ethics in Research Committee (ERC) according to letter 1.163.126; the study was conducted prospectively, with 69 volunteer children (31 girls and 38 boys) with an average age of 8 years and 5 months, with corrected CLP and, with the approval of the ERC, under opinion 2.967.393, with 34 children (16 girls and 18 boys), without CLP, with an average age of 8 years and 6 months.

Participants

In total, we randomly selected 483 children with some type of CLP,\(^22\) with palate-related issues, with or without Simo-nart band, without related syndromes, of both genders, who were undergoing treatment at a hospital specialized in craniofacial anomalies. Appointments with all of them were scheduled individually to verify the possibility that they would participate in the study and undergo the proposed exams, as well as other examinations. A total of 414 patients were excluded, as 250 of them were unable to perform the audiological evaluation, and 164 did not attend the scheduled appointment. Thus, 69 participants with CLP (71% with transforaminal CLP, 19% with pre- and postforaminal CLP, and 10% with postforaminal CLP) were included in the study. To select children without CLP, a partnership was made with a university, in view of the difficulty to find children without such congenital malformation in the routine care of the hospital where the study took place. In total, 41 children were randomly selected through an invitation sent to the people from the community, 7 of which were excluded, as 6 did not complete the evaluation procedures due to the need to leave before the end of the service, and 1 had a mild degree of conductive hearing loss, and was referred for evaluation with an otolarinologist. Thus, the CG was composed of 34 children.

Certain inclusion criteria were the same for the children in both the EG and CG: being between 6 and 11 years old; having hearing thresholds of up to 15dBHL (assessed by pure-tone audiometry) and tympanometry with normal middle ear function; no difficulty in understanding the test to be applied; no hearing complaint and/or affection of the upper airways on the day of the exam (collected from the otolarinological evaluation in the medical record); and no history of complaints of inattention (reported...
saliva collection, to verify its interference in the level of cortisol found.

- **Table 1** shows the general distribution of both groups.

**Procedures**

All children who met the inclusion criteria were first submitted to saliva collection and then to the Sustained Auditory Attention Ability Test (SAAAT).

**Saliva Collection**

Saliva samples were obtained by the researchers and by a dental surgeon, a collaborator in the study, using the Salivette
(Sarstedt, Rommelsdorf, Germany) collection device, in a leveled and standardized way, following the same protocol, and always in the afternoon (between 1:30 p.m. and 5 p.m.), when cortisol levels are relatively stable. The children who had had small amounts of food or liquid within 30 minutes before the collection were asked to rinse with water and wait 3 to 5 minutes. The collection protocol was established: 1) explain to the guardians and child the procedure for saliva collection; 2) open the lid of the Salivette and remove the cotton swab; 3) insert the swab in the child’s mouth, more precisely in the sublingual region; 4) ask the child to wait two to three minutes with the swab in the mouth (no need of chewing simulation); 5) remove the swab from the child’s mouth; 6) store the swab in the plastic device and cover it; and 7) store the samples in styrofoam with ice and take them as soon as possible for storage in the laboratory, at 20°C. For the EG, the cortisol present in the saliva samples was measured using an enzyme immunoassay kit (Salimetrics, Stage College, PA, US), at the Pharmacology and Genetics Laboratory of the institution in which the study was performed; and, for the CG, by a Biomedical in a laboratory of the Biological Sciences Sector. Cortisol levels were determined according to the manufacturer’s instructions, following the standard curve. The detection limits for the age group studied vary from a minimum of 0.1 µg/dL to 0.7422727 µg/dL. The level of salivary cortisol was measured in µg/dL, the unit of measurement used by the manufacturer, although there are studies that use nmol/L (1 µg/dL is equivalent to 27.59 nmol/L). The enzyme immunoassay kit used had sensitivity to detect cortisol levels between 0.007 and 3,000 µg/dL. For the EG, the number of outpatient visits performed on the day of the proposed evaluation was computed, before saliva collection, to verify its interference in the level of salivary cortisol obtained.

**SAAAT**

After saliva collection, the SAAAT was performed, which is a test that assesses the ability to maintain attention and focus on a task for an extended period of time. The SAAAT consists of the presentation of a list of 100 monosyllabic words, recorded in a male voice and presented in the proportion of one word per second. This list (recorded on a CD) was presented six times without interruption. The SAAAT was performed in an acoustic booth, using headphones, with the aid of a CD player coupled to a two-channel audiometer, at an intensity of 50 dBNS, considering the average of the auditory air thresholds for each ear. The test was presented in a binaural and diotic way, and had an average duration of 11 minutes. The evaluator instructed the child orally that they would hear a list of words and should raise their hand whenever they heard the word “no.” To determine the result

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**Table 1** Distribution of the study groups according to age and gender

| Age (years) | Control group (n) | Experimental group (n) |
|-------------|-------------------|------------------------|
|             | Male | Female | Total | Male | Female | Total |
| 6           | 3    | 2      | 5      | 7    | 6      | 13    |
| 7           | 2    | 3      | 5      | 3    | 0      | 3     |
| 8           | 4    | 1      | 5      | 9    | 4      | 13    |
| 9           | 4    | 2      | 6      | 8    | 7      | 15    |
| 10          | 2    | 7      | 9      | 10   | 12     | 22    |
| 11          | 3    | 1      | 4      | 2    | 1      | 3     |
| Total       | 18   | 16     | 34     | 39   | 30     | 69    |
of the SAAAT, the total error score and vigilance decrement were considered. Two types of responses from the child were considered to be errors: inattention, when the child did not raise their hand in response to the target word “no;” and impulsivity, when the child raised their hand to another word instead of the word “no.” A count of the number of inattention errors plus the number of impulsivity errors resulted in the total error score on the SAAAT. The vigilance decrement was obtained from the difference between the number of correct answers given for the word “no” in the first presentation of the list and the number of correct answers obtained in the sixth presentation.

The normality test applied was the Shapiro-Wilk test, and the groups did not present a normal distribution. Thus, we used the median, and the 25th and 75th percentiles. Descriptive statistics were performed for all variables studied in both groups. The Mann-Whitney test was used to compare the groups. The correlation of the level of salivary cortisol with the SAAAT was assessed by the Spearman correlation. A significance level of 5% was adopted.

Results

The characteristics of the study sample are presented in ►Table 1. Of the 483 children with CLP initially selected, 69 were included in the study, and in the CG, of the 41 children invited to participate, 34 were included. Details regarding the recruitment are reported in ►Fig. 1.

Level of Salivary Cortisol

The cortisol levels found in the EG ranged from 0.01567634 µl/dL to 0.1921268 µl/dL, with a median of 0.036 µl/dL; in the CG, it ranged from 0.01 µl/dL to 0.7422727 µl/dL, with a median of 0.180 µl/dL.

►Table 2 shows the median levels of salivary cortisol according to age.

The Mann-Whitney test demonstrated a statistically significant difference when comparing the levels of cortisol \((p = 0.000)\) between the groups, specifically at the ages of 9 \((p = 0.000)\) and 10 \((p = 0.000)\) years.

In both groups, no statistical significance was found regarding gender and levels of cortisol (EG: \(p = 0.412\); CG: \(p = 0.506\)). One to six outpatient visits were performed by the EG, before saliva collection, on the day proposed for the evaluation. The mean daily cortisol levels ranged from 0.048 ± 0.034 µl/dL to 0.055 ± 0.039 µl/dL. A positive correlation \((1,000 p < 0.05)\) between the number of visits and the levels of cortisol was found only for the 7-year-old children in this group.

Correlation between Salivary Cortisol and the SAAAT

Statistical significance was demonstrated by the Spearman correlation between the level of salivary cortisol and the SAAAT regarding the vigilance decrement \((p = 0.010)\), but only in the CG \((n = 34)\) (►Table 5).

Discussion

Salivary Cortisol

The difference between the cortisol levels obtained in this study with those of other studies\(^26,27\) may be due to differences that occurred during the analysis of saliva. The analysis kits are ultra sensitive, and aspects that might seem trivial, such as brightness and temperature, can change the final result. A study conducted\(^27\) with an enzyme immunoassay...
| Age (years) | Experimental group (n = 69) | Control group (n = 34) | p-value |
|------------|----------------------------|------------------------|---------|
|            | Median 25% 75%             | Median 25% 75%         |         |
| Inattention|                           |                        |         |
| 6          | 11.00 5.50 27.00           | 23.00 21.50 33.50      | 0.117   |
| 7          | 19.00 9.00 47.00           | 8.00 6.50 22.50        | 0.250   |
| 8          | 10.00 2.00 17.00           | 8.00 4.50 15.00        | 0.924   |
| 9          | 9.00 2.00 13.00            | 8.50 4.00 17.75        | 0.470   |
| 10         | 4.00 2.00 8.25             | 5.00 1.50 15.00        | 0.749   |
| 11         | 3.00 2.00 12.00            | 5.00 1.25 8.75         | 0.629   |
| Total      | 8.00 2.50 14.00            | 8.00 4.00 17.50        | 0.302   |
| Impulsivity|                           |                        |         |
| 6          | 6.00 3.00 8.50             | 6.00 3.50 12.00        | 0.775   |
| 7          | 4.00 3.00 4.00             | 5.00 3.50 11.50        | 0.250   |
| 8          | 3.00 2.50 9.50             | 2.00 1.50 6.50         | 0.208   |
| 9          | 4.00 2.00 6.00             | 3.50 0.75 7.25         | 0.910   |
| 10         | 2.50 1.00 5.25             | 2.00 0.50 5.50         | 0.781   |
| 11         | 5.00 4.00 9.00             | 3.00 3.00 3.00         | 0.057   |
| Total      | 4.00 2.00 6.00             | 3.00 2.00 6.00         | 0.669   |
| Total error score |           |                        |         |
| 6          | 21.00 11.50 30.50          | 37.00 25.50 41.00      | 0.095   |
| 7          | 23.00 13.00 50.00          | 12.00 10.50 34.00      | 0.393   |
| 8          | 14.00 6.50 23.50           | 15.00 7.00 18.00       | 0.775   |
| 9          | 11.00 7.00 19.00           | 11.50 6.50 23.50       | 0.850   |
| 10         | 7.50 4.75 13.25            | 6.00 3.50 18.50        | 0.881   |
| 11         | 7.00 7.00 21.00            | 8.00 4.25 11.75        | 0.629   |
| Total      | 12.00 7.00 21.00           | 12.00 6.00 24.00       | 0.656   |
| Vigilance decrement |        |                        |         |
| 6          | 2.00 0.50 3.00             | 0.00 –3.00 6.50        | 0.633   |
| 7          | 1.00 –1.00 4.00            | –1.00 –2.00 3.00       | 0.571   |
| 8          | 0.00 1.00 4.00             | –1.00 –1.00 3.50       | 0.095   |
| 9          | 0.00 0.00 2.00             | 0.00 –0.25 3.50        | 0.970   |
| 10         | 0.00 0.00 2.00             | 0.00 0.00 2.00         | 0.915   |
| 11         | 1.00 0.00 1.00             | 0.00 –0.75 2.25        | 0.629   |
| Total      | 1.00 0.00 2.50             | 0.00 –1.00 2.00        | 0.051   |
| Salivary cortisol |         |                        |         |
| 6          | 0.03 0.02 0.05             | 0.39 0.06 0.58         | 0.075   |
| 7          | 0.02 0.02 0.19             | 0.14 0.06 0.27        | 0.571   |
| 8          | 0.04 0.03 0.07             | 0.06 0.01 0.30        | 0.775   |
| 9          | 0.03 0.03 0.08             | 0.22 0.17 0.33        | 0.000*  |
| 10         | 0.03 0.02 0.05             | 0.17 0.14 0.20        | 0.000*  |
| 11         | 0.02 0.02 0.03             | 0.15 0.03 0.20        | 0.400   |
| Total      | 0.03 0.02 0.05             | 0.18 0.11 0.24        | 0.000*  |

Abbreviations: 25%, 25th percentile; 75%, 75th percentile; SAAAT, Sustained Auditory Attention Ability Test.
Notes: Statistical test: Mann-Whitney; *statistical significance.
Table 3  Median, percentiles, comparison of errors (inattention, impulsivity and total score), and vigilance decrement obtained on the SAAAT, and level of salivary cortisol (µl/dL) according to age and gender in the experimental group.

| Age (years) | Male (n = 39) |  | Female (n = 30) |  |  | p-value |
|-------------|---------------|---|----------------|---|---|--------|
|             | Median | 25% | 75% |        | Median | 25% | 75% |        |
| **Inattention** |       |       |       |        |       |       |       |        |
| 6           | 11.00  | 6.00 | 24.00 | 12.00  | 4.00  |      | 35.75 |
| 7           | 28.00  | 6.75 | 35.26 | 19.00* | "     |      | "     |
| 8           | 10.00  | 4.50 | 17.50 | 6.00   | 1.25  |      | 16.75 |
| 9           | 3.00   | 1.25 | 15.25 | 9.00   | 6.00  |      | 13.00 |
| 10          | 4.00   | 2.00 | 9.25  | 4.00   | 2.25  |      | 8.50  |
| 11          | 2.50   | 1.50 | 2.26  | 12.00* | "     |      | "     |
| Total       | 7.50   | 2.00 | 15.50 | 8.00   | 3.00  |      | 13.00 | 0.885 |
| **Impulsivity** |       |       |       |        |       |       |       |        |
| 6           | 8.00   | 4.00 | 10.00 | 4.00   | 1.75  |      | 7.25  |
| 7           | 3.50   | 2.25 | 3.01  | 4.00   | "     |      | "     |
| 8           | 4.00   | 3.00 | 13.50 | 2.50   | 1.25  |      | 5.25  |
| 9           | 3.50   | 2.25 | 6.00  | 4.00   | 2.00  |      | 4.00  |
| 10          | 4.50   | 1.00 | 5.50  | 1.00   | 0.25  |      | 5.75  |
| 11          | 4.50   | 3.00 | 3.76  | 9.00   | "     |      | "     |
| Total       | 4.00   | 3.00 | 7.25  | 3.00   | 1.00  |      | 6.00  | 0.030**|
| **Total error score** |       |       |       |        |       |       |       |        |
| 6           | 27.00  | 12.00 | 27.00 | 15.50  | 8.75  |      | 38.25 |
| 7           | 31.50  | 9.75 | 37.51 | 23.00  | "     |      | "     |
| 8           | 16.00  | 8.00 | 26.50 | 10.00  | 3.50  |      | 19.50 |
| 9           | 8.00   | 4.75 | 20.50 | 12.00  | 10.00 |      | 19.00 |
| 10          | 7.50   | 4.75 | 14.25 | 7.50   | 3.50  |      | 11.50 |
| 11          | 7.00   | 5.25 | 5.26  | 21.00  | "     |      | "     |
| Total       | 13.00  | 7.00 | 25.50 | 11.00  | 7.00  |      | 19.00 | 0.375 |
| **Vigilance decrement** |       |       |       |        |       |       |       |        |
| 6           | 1.00   | 0.00 | 3.00  | 3.00   | 0.75  |      | 5.25  |
| 7           | 0.00   | -0.75| 0.76  | 4.00   | "     |      | "     |
| 8           | 1.00   | 0.50 | 3.50  | 2.00   | 0.00  |      | 5.50  |
| 9           | 0.00   | -0.75| 1.75  | 0.00   | 0.00  |      | 3.00  |
| 10          | 0.50   | 0.00 | 2.00  | 0.00   | -0.75|      | 1.00  |
| 11          | 0.50   | 0.00 | 0.76  | 1.00   | "     |      | "     |
| Total       | 1.00   | 0.00 | 2.00  | 1.00   | 0.00  |      | 3.00  | 0.743 |
| **Salivary cortisol** |       |       |       |        |       |       |       |        |
| 6           | 0.03   | 0.02 | 0.08  | 0.03   | 0.02  |      | 0.04  |
| 7           | 0.02   | 0.01 | 0.02  | 0.19   | "     |      | "     |
| 8           | 0.04   | 0.03 | 0.06  | 0.05   | 0.03  |      | 0.12  |
| 9           | 0.05   | 0.03 | 0.09  | 0.03   | 0.02  |      | 0.03  |
| 10          | 0.04   | 0.03 | 0.06  | 0.03   | 0.02  |      | 0.05  |
| 11          | 0.02   | 0.01 | 0.03  | 0.03   | "     |      | "     |
| Total       | 0.04   | 0.02 | 0.06  | 0.03   | 0.02  |      | 0.05  | 0.412 |

Abbreviations: 25%, 25th percentile; 75%, 75th percentile; SAAAT, Sustained Auditory Attention Ability Test. Notes: Statistical test: Mann-Whitney; *Only one individual in this age group and of that gender; **statistical significance.
Table 4: Median, percentiles, comparison of errors (inattention, impulsivity and total score), and vigilance decrement obtained on the SAAAT, and level of salivary cortisol (µl/dL) according to age and gender in the control group.

| Age (years) | Male (n = 18) | Female (n = 16) | p-value |
|-------------|---------------|-----------------|---------|
|              | Median  | P25%  | 75%    | Median  | 25%  | 75%    |         |
| Inattention |        |       |        |        |       |        |         |
| 6           | 23.00  | 22.00 | 38.00  | 25.00  | 15.75 | 21.75  |         |
| 7           | 19.50  | 5.25  | 24.09  | 8.00   | 6.00  | 13.00  |         |
| 8           | 10.50  | 3.50  | 16.00  | 7.00*  | *     | *      |         |
| 9           | 11.00  | 5.50  | 29.25  | 5.50   | 3.00  | 5.30   |         |
| 10          | 8.00   | 2.25  | 9.79   | 5.00   | 0.00  | 17.00  |         |
| 11          | 8.00   | 2.00  | 9.00   | 1.00*  | *     | *      |         |
| Total       | 11.00  | 6.25  | 22.25  | 6.50   | 4.00  | 16.00  | 0.117   |
| Impulsivity |        |       |        |        |       |        |         |
| 6           | 6.00   | 2.00  | 15.00  | 7.00   | 3.75  | 6.75   |         |
| 7           | 7.50   | 2.25  | 9.09   | 5.00   | 4.00  | 11.00  |         |
| 8           | 2.00   | 1.25  | 2.75   | 10.00* | *     | *      |         |
| 9           | 3.50   | 1.50  | 9.25   | 3.00   | 0.00  | 4.55   |         |
| 10          | 4.50   | 1.50  | 5.29   | 1.00   | 0.00  | 5.00   |         |
| 11          | 3.00   | 3.00  | 3.00   | 3.00*  | *     | *      |         |
| Total       | 3.00   | 2.00  | 6.25   | 4.50   | 1.00  | 6.00   | 0.878   |
| Total error score |        |       |        |        |       |        |         |
| 6           | 37.00  | 25.00 | 44.00  | 32.00  | 19.50 | 28.50  |         |
| 7           | 27.00  | 7.50  | 33.09  | 12.00  | 11.00 | 24.00  |         |
| 8           | 13.00  | 5.00  | 18.00  | 17.00* | *     | *      |         |
| 9           | 14.50  | 7.00  | 38.50  | 8.50   | 5.25  | 7.55   |         |
| 10          | 12.50  | 3.75  | 15.04  | 6.00   | 2.00  | 17.00  |         |
| 11          | 11.00  | 5.00  | 12.00  | 4.00*  | *     | *      |         |
| Total       | 14.00  | 8.75  | 28.00  | 10.50  | 6.00  | 22.25  | 0.281   |
| Vigilance decrement |        |       |        |        |       |        |         |
| 6           | 6.00   | −3.00 | 7.00   | −1.50  | −2.25 | 0.00   |         |
| 7           | −2.00  | −2.25 | −0.66  | 2.00   | −1.00 | 4.00   |         |
| 8           | −0.50  | −1.00 | 5.25   | −1.00* | *     | *      |         |
| 9           | 1.00   | −0.75 | 6.50   | 0.00   | 0.00  | 0.05   |         |
| 10          | 0.00   | 1.00  | 1.54   | 0.00   | 0.00  | 2.00   |         |
| 11          | 0.00   | −1.00 | 3.00   | 0.00*  | *     | *      |         |
| Total       | 0.00   | −1.00 | 3.75   | 0.00   | −0.75 | 1.50   | 0.798   |
| Salivary cortisol |        |       |        |        |       |        |         |
| 6           | 0.42   | 0.11  | 0.74   | 0.20   | 0.00  | 0.29   |         |
| 7           | 0.06   | 0.00  | 0.17   | 0.20   | 0.14  | 0.34   |         |
| 8           | 0.13   | 0.02  | 0.35   | 0.01   | *     | *      |         |
| 9           | 0.28   | 0.20  | 0.40   | 0.17   | 0.12  | 0.18   |         |
| 10          | 0.14   | 0.09  | 0.15   | 0.20   | 0.15  | 0.21   |         |
| 11          | 0.18   | 0.12  | 0.21   | 0.01   | *     | *      |         |
| Total       | 0.18   | 0.11  | 0.32   | 0.17   | 0.04  | 0.20   | 0.506   |

Abbreviations: 25%, 25th percentile; 75%, 75th percentile; SAAAT, Sustained Auditory Attention Ability Test. Notes: Statistical test: Mann-Whitney; *Only one individual in this age group and of that gender.
The present research (the manufacturer, were well above the results obtained in comparison with the parameters described in the present research (Table 2). The results of the present study were within the parameters established by the manufacturer (up to 0.21 µg/dL) for the age range studied. Differences in cortisol metabolism between male and female participants are already present in childhood, with a higher level of salivary cortisol in boys older than 8 years of age compared with girls. However, in the present study, although the salivary cortisol was slightly higher among boys, the absence of statistical significance (p = 0.41 and p = 0.50 respectively, for the EG and CG) regarding cortisol levels between boys and girls corroborate the findings of other studies, which also showed no significant differences in cortisol levels in the results obtained with children of different genders.

The place where the study tests were performed, a room within the hospital, could justify the statistically significant difference in the increase in the level of cortisol in the CG in relation to the EG. Thus, the place and event, strange to the child, can trigger reactions of insecurity, fear of the unknown, and cause anxiety and stress. Abnormal activity in the HPA axis has been observed in patients with anxiety. A study showed that the levels of salivary cortisol just before the performance of the medical procedure were positively correlated with the anxiety of the participants, and the levels of salivary cortisol and anxiety were associated with increased stress. However, the testing site was not an unknown environment for children with CLP, in view of their constant and contact with the health professionals—who provide different and humanized care in the hospital, which would explain the absence of high levels of salivary cortisol in most of the EG. Taking into account the number of outpatient visits before the saliva collection for the EG, the same thing can be considered. It is noteworthy, however, that the significant correlation found only for the 7-year-old EG children may be explained by the fact that this group, at this age, was formed by only 3 individuals. In addition, one of the children had the highest level of salivary cortisol (0.199 µg/dL), which may have interfered with the median cortisol in this group as a whole. In a study that associated the stress response through cortisol and a health-related quality of life questionnaire (KIDSCREEN-52), performed with the participation of children with CLP aged 5 to 10 years and a control group paired by age, the authors found that there was no difference in cortisol concentrations between children with different types of cleft. The study concluded that children with CLP did not show a higher level of stress compared with the control group.

### SAAAT

In the present study, the ability to sustain auditory attention was verified by the total score of errors (inattention + impulsivity) and the vigilance decrement, obtained with the application of the SAAAT in both groups, without hearing or attention complaints, and without hearing impairment at the time of the assessment.

The results of the present study (Tables 3 and 4) are in agreement with those of other studies that showed that, in continuous-performance tests, such as the SAAAT, age has a greater influence than gender. In the EG (Table 3), when comparing the age groups of 6, 7 and 8 years, with 9, 10 and 11 years, this finding can be verified. However, the 7-year-old children in the EG showed worse results than the 6-year-old children, as well as the 11-year-olds in relation to the 10-year-olds. It is noteworthy that, in the present study, the 7-year-old children with CLP, when presenting a large number of inattention errors, contributed to the increase in the total error score, determining the age that had the worst performance. Once again, attention is drawn to the fact that a small number of children, only three, were of this age, an aspect that may contribute to this finding. For the CG, younger children scored higher (worse) regarding the total error score, except for the 9-year-olds (Table 4), who had a higher score than the 8-year-olds. This result is due to the greater number of inattention and impulsivity errors, when compared with those of the 8-year-olds. However, a study that used the same instrument to assess sustained auditory

### Table 5 Correlation coefficient between the performance on the SAAAT and the level of cortisol

| Cortisol | Total error score | Vigilance decrement | Inattention | Impulsivity |
|----------|------------------|---------------------|-------------|-------------|
| **Experimental group** | | | | |
| r        | 0.09             | 0.02                | 0.07        | 0.07        |
| p-value  | 0.444            | 0.840               | 0.537       | 0.566       |
| N        | 69               | 69                  | 69          | 69          |
| **Control group** | | | | |
| r        | 0.17             | 0.43                | 0.21        | 0.02        |
| p-value  | 0.320            | 0.010*              | 0.214       | 0.874       |
| N        | 34               | 34                  | 34          | 34          |

**Abbreviation:** SAAAT, Sustained Auditory Attention Ability Test.

**Notes:** Statistical test: Spearman correlation; * statistical significance.
attention in 50 children aged 7 years without CLP concluded that the children evaluated in the afternoon and those studying in public schools had a worse performance in the ability to sustain auditory attention, showing that the period of the day and the type of school can be a possible source of variation in the child’s ability to sustain auditory attention. Following the methodology applied in a previous study, this study was applied in the afternoon, but the type of school (public or private) was not investigated in children with or without CLP. In this study, regarding the value obtained for the vigilance decrement, the other parameter considered in the performance on the SAAAT, the older children in both groups showed scores within normal values for all ages evaluated. Other researchers have reported that, for the vigilance decrement, 7-year-olds with CLP had a worse performance on the SAAAT than those without CLP. In this study, although the boys in most of the age groups tested showed a more inattentive and impulsive behavior (verified in the values of the errors made on the test), and the girls in most age groups tested had a better performance (verified in the total error score), the differences found between genders in both groups were not significant. This was also reported in other studies that used the SAAAT. Some studies reported significantly higher scores on inattention in boys with CLP compared with the controls. In the present study, the impulsivity error was significant \((p = 0.030)\) among the male children in the EG. Difficulties related to inhibitory control are generally associated with impulsivity, found in tasks of continuous performance, and are called commission errors, that is, when the subject responds to a stimulus that was not the specified target. In continuous-performance tasks, when the impulsive action is measured as an absolute number of inhibitory failures, males show greater impulsivity, according to a study. Another study reports that boys with isolated CLP have higher levels of hyperactivity, impulsivity and inattention compared with controls, and this is directly related to a significant increased volume of the right ventromedial prefrontal cortex, the region of the brain involved in impulse control behaviors.

A significant difference was expected in the SAAAT results of both groups, considering that, in the population with craniofacial anomalies, especially in those with CLP, the high occurrence of middle-ear alterations is well known, with consequent conductive hearing loss. Authors suggest in their study that the main aspects of auditory and cognitive skills are impaired by the minimal and mild, symmetrical and asymmetrical hearing loss. Also, long periods of sensory deprivation, in addition to conductive hearing loss, can lead to perceptual hearing deficits and interfere in the development of the structures and functions of the central nervous system. It is noteworthy that, in the questionnaire applied to the parents or guardians of the children with and without CLP, in this study, almost 41% reported the presence of otitis media during early childhood. Moreover, a study demonstrated that the SAAAT performance of 7-year-old children with a type of CLP similar to the one herein analyzed was inferior to that of children without CLP.

**Correlation between Salivary Cortisol and the SAAAT**

The statistical significance obtained in the correlation between the levels of salivary cortisol and vigilance decrement in the CG \((\text{- Table 5})\), showing that the higher the level of salivary cortisol, the more the attention of these children declined during the test. It is noteworthy, however, that the values of salivary cortisol and vigilance decrement were within the normal range for both groups. The absence of a correlation between the level of cortisol and the quality of life of children with CLP has also been demonstrated in a previous research.

The present study makes some considerations that must be observed in future trials, such as the fact that obtaining a more homogeneous sample with a larger number of individuals may provide more accurate information about the correlation between the level of cortisol and sustained auditory attention. The application of questionnaires to assess anxiety and obtain data on quality of life may contribute with additional information on stress and increased levels of cortisol. It would also be interesting to conduct a study to verify the impact of the hospital environment on the child’s anxiety, with double collections, using the same child as a control, and comparing the samples collected in a familiar environment with the ones obtained in the hospital environment.

A limitation of the present study is that we did not apply instruments to assess the socioeconomic characteristics and/or psychological factors that could affect the stress level of children, focusing only on the laboratory analysis of the level of cortisol and the ability to sustain auditory attention; the evaluation of these characteristics is suggested in future studies.

**Conclusions**

All children had median levels of salivary cortisol and scores for sustained auditory attention within normal parameters. A significant correlation between the level of salivary cortisol level and the ability to sustain auditory attention was observed in the CG. There was no difference regarding the performance on the SAAAT and the level of salivary cortisol between female and male subjects.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

Acknowledgments to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grant for the development of this study – Grant No. 306750/2015-5.

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