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# Association between salivary cortisol level and caries in early childhood

## ABSTRACT

**Aim** The present study aimed to evaluate the association between caries and oral health status, age, salivary cortisol levels, and parental education in children with and without prior dental caries experience.

**Methods** An observational case-control study was performed including 122 children aged between 3 and 6 years who were clinically examined for caries experience using the sum of decayed, missing, and filled teeth in the primary (dmft index) and permanent (DMFT index) dentition. Oral health status was also evaluated using the Simplified Oral Hygiene index (OHI-S). Parents filled a questionnaire to provide information on other variables. Salivary cortisol levels were estimated 1 h after routine dental brushing.

**Results** We found that dental caries experience was associated with cortisol level, plaque, age, and high calculus levels. High cortisol levels and age are important risk factors for caries development with odds ratios of 3.05 (95% CI: 1.84–5.06) and 1.59 (95% CI: 1.09–2.58), respectively. Multivariate logistic analysis showed that cortisol level and age were independently associated with caries presence. Caries experience was not associated with education of parents, feeding-hygiene habits of child or birth events.

**Conclusion** The present findings support the hypothesis that caries is mainly correlated with high salivary cortisol levels. Dental caries experience in children was also positively associated with tartar, plaque, and age.

**Keywords** Cariology; Dental health; Pediatric dentistry; Saliva.

## Introduction

Bacterial fermentation of food debris on a tooth surface results in acid production, which can cause demineralisation and destruction of hard tissues of the teeth—termed dental caries. [Selwitz et al., 2007].

This disease requires primary aetiological actors, including a host, a dietary substrate, and bacteria. Caries formation may also be influenced by secondary factors, such as diet, oral hygiene, and salivary composition and flow rate. Studies have also suggested that dental caries is correlated with anxiety and personality [Morse et al., 1983; Klages et al., 2005; Barbosa et al., 2012]. Children and adults with dental caries have been reported to show several predisposing factors, including repressed emotions and fears, dissatisfaction with achievement, feelings of inferiority, rebellion against a home situation, traumatic school experiences, continuous general tension and anxiety, emotional disturbances, and other factors related to the socioeconomic status [Rai et al., 2010].

Cortisol, a hormone secreted by the hypothalamus pituitary adrenal axis, is used as a stress biomarker in both adults and children, and salivary cortisol levels are considered a reliable, accurate, and noninvasive measure of stress [Jessop et al., 2008; Hellhammer et al., 2009]. Salivary cortisol level has been used to assess the role of stress in a variety of settings, including anxiety regarding dental treatment, periodontal disease, and dental caries [Rai et al., 2010; Blomqvist, 2010; Rai et al., 2011; Yfanti et al., 2014].

Pani et al. [2013] used a biomarker to demonstrate the correlation between parent stress levels and child stress levels in relation to early childhood caries, asserting that stress of the parents (especially the mother) could be transmitted to their children.

Boyce et al. [2010] found positive associations among socioeconomic status and family financial stressors, dental caries, and basal salivary cortisol secretion in children in response to a stressor.

Rai et al. [2010] reported that children with rampant caries exhibited increased salivary cortisol levels, which then decreased after three months of dental treatment. They proposed that children show a reduced ability to cope with stress when they have greater dental caries experience.

Conversely, Kambalimath et al. [2010] reported that children with and without caries showed the same coping ability and levels of stress produced by different dental procedures.

Due to this controversy in the literature, further

studies are needed to clarify the relationship between stress and dental caries. It is reasonable to think that the interaction of cortisol and bacteria modifies caries risk, as acute and chronic stressors can impair IgA production [Deinzer et al., 2000; Phillips et al., 2006], cortisol can affect local mucosa immunity and oral microbial flora [Genco, 1998] and mucosa immune competence influences bacterial colonisation and growth [Kamma, 1998; Nogueira et al., 2005].

The present study aimed to evaluate how caries presence was associated with oral health status, age, salivary cortisol levels, and parental education among children with and without dental caries experience.

## Materials and methods

### Study design and subjects

We performed an observational case-control study including 122 children with dental caries experience ( $n = 58$ ) or without dental caries experience ( $n = 64$ ). Eligible children aged between 3 and 6 years, and were recruited from the Pediatric Dental Clinic of the University of L'Aquila (Italy), between October 2013 and March 2014. Children were excluded if they had any systemic or congenital disease, or were using any medications during the saliva collection period. Participants were also excluded if they did not collect saliva or submitted insufficient/contaminated samples.

This study was approved by the Ethics Committee of the University of L'Aquila (Italy) (Prot. N29579 - 06/09/2013). Prior to patient enrolment, we explained the study methodology and aim, and the participants' parents or legal guardians completed informed consent forms and questionnaires. The questionnaires were specially designed to collect relevant information regarding age, gender, and various caries risk factors—namely, parent education, feeding-hygiene habits, birth events, and medical condition.

### Patient examination

Prior to study initiation, a 20-hour calibration process was performed. This process included a theoretical discussion of the examination technique to be applied; revision of the number of decayed, missing, and filled teeth (DMFT) evaluated by dmft criteria; and slide presentations of various conditions that might be clinically observed. The calibration process also included a clinical exercise intended to provide an environment for learning the previously acquired theoretical information.

Two examiners performed the examinations of 122 children. Caries experience was assessed using a visual/tactile method with a dental probe and mirror, with the subject on a dental chair and under a dental light unit, to determine the counts of decayed, missing, and filled dental surfaces (DMFT, following the WHO

criteria) [Petersen et al., 2008].

A dental explorer was gently used on the dental surfaces to clean the area before examination, as well as to evaluate the surface texture (degree of surface roughness). Non-cavitated lesions were identified when the brushed and air-dried surfaces of the tooth presented a 'chalky-white' appearance. Teeth were considered decayed when at least one surface presented clinical signs of cavitated or white spot lesions. Bitewing radiographs were taken to detect proximal caries.

For all children, oral health status was assessed using the Simplified Oral Hygiene index (OHI-S) as described by Greene and Vermillion, and its modification for deciduous teeth by Miglani et al. [Greene et al., 1964; Miglani et al., 1973]. These indices assess the extent of two local irritative factors—plaque and tartar—considering plaque extension, not thickness. The OHI-S requires evaluation of the buccal surfaces of six teeth, indicating the extent of plaque or soft deposits (DI), and tartar or hard deposits (CI). The variables of tartar and plaque were calculated using four levels—no presence, 1/3 of the tooth surface, 2/3 of the tooth surface, total coverage—which was an ideal system for quantitative assessment, allowing quick evaluation of disease extent and spread.

After the evaluation of these criteria, participants were separated into two different groups: children with dental caries experience (CE; DMFT  $\geq 1$ ;  $n = 58$ ) and those who were caries-free (CF; DMFT = 0;  $n = 64$ ).

### Saliva collection

Whole unstimulated saliva samples were collected 1 hour after routine dental brushing, between 4 pm and 6 pm for all children. During the 60 minutes prior to saliva collection, children were instructed to avoid eating or drinking caffeine-containing beverages or fruit juices. Children were seated with their head slightly down, and were asked not to swallow or move their tongue or lips during the collection period. Saliva was allowed to accumulate in their mouth for 2 minutes, and then the children were asked to spit the accumulated saliva into an ice-cooled tube.

### Analysis of salivary cortisol

Whole unstimulated saliva samples were centrifuged at 3500xg for 20 min. The clarified saliva supernatants were collected and stored at  $-80^{\circ}\text{C}$  for later analysis. Cortisol levels (in ng/ml) were determined in the fluid saliva fraction using an ELISA kit (DRG Salivary Cortisol ELISA; DRG International, Inc., USA) following the manufacturer's instructions.

### Statistical analysis

The population size needed for this study was calculated *a priori*. Using a power calculator, it

| Characteristic                                                                                                                                                                                                                                                                                                                            | Total<br>N = 122 | CF n = 64<br>(52.46%) | CE n = 58<br>(47.54%) | p value |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|-----------------------|-----------------------|---------|
| Gender n (%) <sup>a</sup>                                                                                                                                                                                                                                                                                                                 |                  |                       |                       |         |
| Male                                                                                                                                                                                                                                                                                                                                      | 69 (56.56)       | 36 (52.17)            | 33 (47.83)            | 0.943   |
| Female                                                                                                                                                                                                                                                                                                                                    | 53 (43.44)       | 28 (52.83)            | 25 (47.17)            |         |
| Age in years, median (IQR) <sup>b</sup>                                                                                                                                                                                                                                                                                                   | 5 (4–6)          | 5 (3–6)               | 5 (4–6)               | 0.060   |
| <sup>a</sup> $\chi^2$ test.<br><sup>b</sup> Wilcoxon Mann-Whitney test.<br>p > 0.05, no statistically significant difference between CE and CF.<br>p < 0.05, statistically significant difference between CE and CF.<br>CE, Children with dental caries experience, dmft $\geq$ 1.<br>CF, Children who were dental caries-free, dmft = 0. |                  |                       |                       |         |

**TABLE 1** Distributions of gender and age among children in the CE and CF groups.

was determined that  $\geq 55$  subjects per group were needed in order to demonstrate an association between salivary cortisol level and caries experience. Specifically, we calculated that this sample size would give our study 80% power ( $1-\beta$ ) to detect an absolute between-groups difference in cortisol concentration of approximately 21% with a 10% standard deviation (which was considered clinically relevant) using a two-sided test with  $\alpha = 0.05$ . Cortisol level was used as the main response variable.

Descriptive analyses were performed to illustrate the characteristics of the CE and CF groups. Frequencies and percentages were calculated for discrete and nominal values, while quantitative variables were evaluated using median and interquartile range (IQR). The normality of data distribution was evaluated using the Kolmogorov-Smirnov test. As the data were not normally distributed, non-parametric analyses were used to determine between-group differences. The Wilcoxon Mann-Whitney test was used to examine differences between mean values for demographic and clinical variables. Frequency differences between the groups were calculated using the  $\chi^2$  test or Fisher's exact test. The  $\chi^2$  test was used to evaluate the statistical significance of between-group differences in rising levels (ordinal variable) of tartar and plaque.

We used univariate and multivariate logistic regression models to estimate the odds ratios (OR) and their 95% confidence intervals (95% CI) for associations between caries presence (considered the dependent variable) and the contribution to this condition of demographic and clinical variables (considered explanatory variables).

The answers given by parents in the anamnestic questionnaire were evaluated and used to analyse the respondents' abilities to perform activities to promote oral hygiene. Choices were made between several predetermined answers posed on a scale of ordinal scores.

Statistical tests were bidirectional and used with a 5% significance level. The data were processed using the statistical package STATA/IC 12.0.

## Results

The sample comprised 122 children, of whom 69 were male (56.56%) and 53 female (43.44%). The median age was 5 years (IQR, 4–6). No significant differences were observed between the two examined groups with respect to gender ( $p = 0.943$ ) and age ( $p = 0.060$ ). The age and gender distribution in CF and CE groups is tabulated in Table 1.

The plaque index significantly differed between the two groups ( $p = 0.014$ ), with a lower percentage of children without plaque in the CE group (28.26%) than in the CF group (71.74%). Plaque on 1/3 of the tooth surface was noticed in 55.81% of the CE group and 44.19% of the CF group, while plaque on 2/3 of the tooth surface was observed in 63.64% of the CE group and 36.36% of the CF group (Fig. 1A). The tartar index also significantly differed between groups ( $p = 0.009$ ), with no tartar observed in 63.64% of the CF group compared to 36.36% of the CE group. Tartar was noted on 1/3 of the tooth surface in 50% of both the CF and CE groups, while tartar on 2/3 of the tooth surface was detected in 33.33% of the CF group vs. 66.67% of the CE group (Fig. 1B). No child exhibited total plaque or total tartar coverage in either group. Cortisol levels in the fluid saliva fraction were significantly higher in the CE group (1.85 ng/ml; IQR, 1.51–2.67 ng/ml) than in the CF group (1.35 ng/ml; IQR, 0.15–1.95 ng/ml) ( $p = 0.0001$ ) (Fig. 2).

To predict the simultaneous association of each independent variable with dental caries, was performed an initial univariate logistic regression analysis (Table 2). The results showed that plaque build-up was a risk factor associated with caries presence, with an OR of 3.18 (95% CI: 1.13–8.91) for low plaque levels and an OR of 4.6 (95% CI: 1.48–14.20) for higher plaque levels. Only high tartar values were associated with caries presence, with an OR of 3.46 (95% CI: 1.08–11.08). High cortisol levels and increased age were also important risk factors for caries, with OR values of 3.05 (95% CI: 1.84–5.06) and 1.59 (95% CI: 1.09–2.58), respectively.

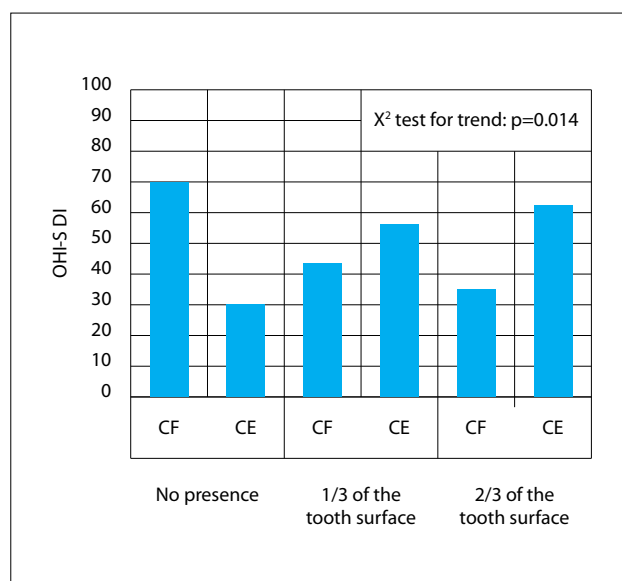


FIG. 1A

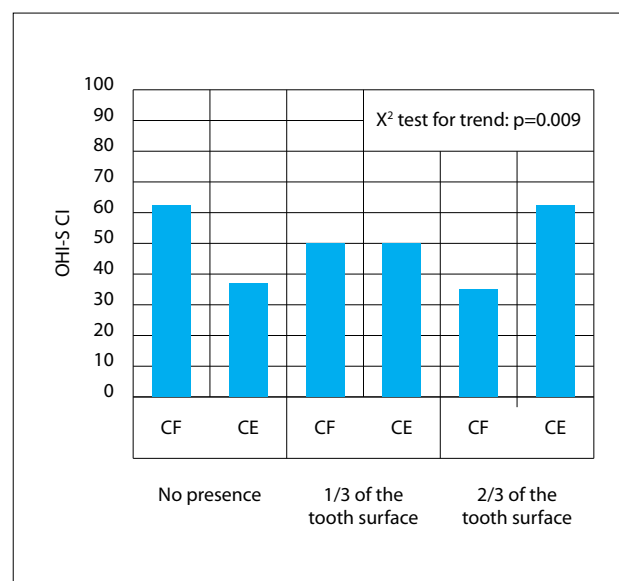


FIG. 1B

**FIG. 1** Distribution of oral health status among children in the CE and CF groups. A) Plaque index (OHI-S DI). B) Tartar index (OHI-S CI).

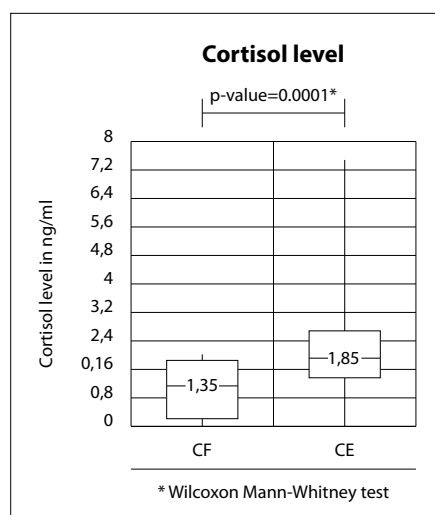


FIG. 2

Distribution of cortisol levels among children in the CE and CF groups.

Parent education was not significantly associated with caries experience.

We corrected the model for the effects of cortisol and age using a multivariate logistic analysis that showed how these associations were maintained. Our results supported the hypothesis that higher cortisol levels and increased age were independently associated with caries presence. The OR values adjusted for age and cortisol levels were 2.33 (95% CI: 1.15–4.70) for higher cortisol level and 1.86 (95% CI: 1.11–3.17) for increased age (Table 3).

Parents of all participating children completed the questionnaire after the observational period. Table 4 shows the answers. The factors assessed by this questionnaire, including feeding-hygiene habits

| Characteristic                | OR   | 95% CI        |
|-------------------------------|------|---------------|
| <b>OHI-S DI (Plaque)</b>      |      |               |
| no presence <sup>#</sup>      | 1    |               |
| 1/3 of the tooth surface      | 3.18 | (1.13–8.91)*  |
| 2/3 of the tooth surface      | 4.6  | (1.48–14.20)* |
| <b>OHI-S CI (Tartar)</b>      |      |               |
| no presence <sup>#</sup>      | 1    |               |
| 1/3 of the tooth surface      | 1.71 | (0.64–4.53)   |
| 2/3 of the tooth surface      | 3.46 | (1.08–11.08)* |
| <b>Age in years</b>           | 1.59 | (1.09–2.58)*  |
| <b>Cortisol level (ng/ml)</b> | 3.05 | (1.84–5.06)*  |
| <b>Education of mother</b>    |      |               |
| Middle School <sup>#</sup>    | 1    |               |
| High school diploma           | 0.79 | (0.27–2.31)   |
| Degree                        | 0.44 | (0.12–1.58)   |
| <b>Education of father</b>    |      |               |
| Middle School <sup>#</sup>    | 1    |               |
| High school diploma           | 1.04 | (0.39–2.77)   |
| Degree                        | 0.55 | (0.15–1.99)   |

<sup>#</sup>Reference category.  
 \*Statistically significant association.  
 OR, odds ratio.  
 95% CI, 95% confidence interval.

**TABLE 2** Univariate logistic regression analysis of association between caries presence with oral health status, age, cortisol level, and parent education.

| Risk factor    | OR   | 95% CI       |
|----------------|------|--------------|
| Age in years   | 1.86 | (1.11–3.17)* |
| Cortisol level | 2.33 | (1.15–4.70)* |

\*Statistically significant association.  
OR, odds ratio.  
95% CI, 95% confidence interval.  
OR adjusted for age and cortisol levels.

**TABLE 3** Multivariate logistic regression analysis of caries probability by age and cortisol level.

and birth events of the child, were not significantly associated with caries presence.

## Discussion

The aim of the present study was to evaluate the association of caries presence with clinical parameters (plaque and tartar), age, salivary cortisol levels, and parent education among children with and without dental caries experience.

In response to a stressful situation, neurotransmitters and hormones released by the hypothalamic-pituitary-adrenal (HPA) system trigger an emotional response of fear, and suppress activity in the brain. Cortisol secretion is considered a key event in HPA axis activation following stress stimuli. Ability to react to a stressor differs based on an individual's personality, physical strength, and health. Psychosocial variables—such as stress, depression, and anxiety—are strongly associated with periodontal diseases, and chronic stress impacts the oral cavity in a variety of ways. Stress can lead to a reduction of salivary flow rate, and corticosteroids cause atrophic changes in the major salivary glands, altering the saliva composition and total volume. Although no study has examined the relationship between salivary cortisol secretion and the physical properties of teeth, exposure to therapeutic corticosteroids can induce hypoplasia [Bublitz A et Al., 1981] which increases dental caries susceptibility [Hong et al., 2009].

Although salivary cortisol levels correspond to 50–60% of plasma cortisol concentrations, salivary cortisol can be a useful marker of adrenocortical function as an index for stress [Vining, 1987; Tahara et al., 2007]. Salivary cortisol assessment is a non-invasive, accurate, and unaltered measurement of non-protein-bound, biologically active, free cortisol [Umeda et al., 1981]. Previous studies have investigated the use of salivary cortisol as a biomarker for stress, and several groups have focused on the connection between cortisol level and early childhood caries (ECC) [Pani SC et Al., 2013; Kambalimath HV et Al., 2010].

However, the relationship between salivary cortisol level and dental caries experience among children

| Question                                                             | CE n = 58  | CF n = 64  | p-value* |
|----------------------------------------------------------------------|------------|------------|----------|
| 1) How many times a day does your child brush his/her teeth?         |            |            |          |
| 1 time                                                               | 19 (31.82) | 22 (36.36) | 0.906    |
| 2 times                                                              | 32 (54.55) | 36 (56.82) |          |
| 3 times                                                              | 7 (11.36)  | 6 (9.09)   |          |
| 2) At what age did your child first start brushing his/her teeth?    |            |            |          |
| Before 1 year                                                        | 5 (9.04)   | 9 (13.64)  | 0.461    |
| 1–2 years                                                            | 38 (65.91) | 38 (59.09) |          |
| 3–4 years                                                            | 12 (20.45) | 17 (27.27) |          |
| 5 years                                                              | 3 (4.55)   | 0 (0.00)   |          |
| Does your child use a fluoride toothpaste?                           |            |            |          |
| No                                                                   | 46 (79.55) | 51 (79.55) | 1.000    |
| yes                                                                  | 12 (20.45) | 9 (20.45)  |          |
| Did your child take drops or tablets containing fluoride?            |            |            |          |
| No                                                                   | 20 (34.09) | 32 (50.00) | 0.130    |
| Yes                                                                  | 38 (65.91) | 32 (50.00) |          |
| Does your child have sweet food or sugary drinks between meals?      |            |            |          |
| No                                                                   | 46 (79.55) | 51 (79.55) | 1.000    |
| Yes                                                                  | 12 (20.45) | 13 (20.45) |          |
| How often does your child eat sweets?                                |            |            |          |
| More than once a day                                                 | 36 (61.36) | 38 (59.09) | 0.947    |
| Several times a week                                                 | 15 (27.27) | 17 (27.27) |          |
| Less than once a week                                                | 7 (11.36)  | 9 (13.64)  |          |
| Was your child born at term with a normal childbirth? s              |            |            |          |
| Yes                                                                  | 29 (65.91) | 31 (70.45) | 0.647    |
| No                                                                   | 15 (34.09) | 13 (29.55) |          |
| Your child was:                                                      |            |            |          |
| breastfed                                                            | 34 (59.09) | 41 (63.64) | 0.201    |
| fed with a bottle                                                    | 9 (15.91)  | 16 (25.00) |          |
| both                                                                 | 15 (25.00) | 7 (11.36)  |          |
| Data are given as n (%).                                             |            |            |          |
| CE, Children with dental caries experience, dmft ≥ 1.                |            |            |          |
| CF, Children who were dental caries-free, dmft = 0.                  |            |            |          |
| * $\chi^2$ test or Fisher's exact test.                              |            |            |          |
| p > 0.05, no statistically significant difference between CE and CF. |            |            |          |

**TABLE 4** Questionnaire responses about feeding-hygiene habits and birth events.

remains unclear. Kambalimath et al., in fact, showed no significant differences between children with caries and children without caries [Kambalimath et al., 2010], whereas Pani and Odhaib [2013] observed that dental treatment significantly reduced the salivary cortisol levels of children with early caries, but the post-treatment levels remained significantly higher than those of caries-free children. Furthermore, Pani et al. demonstrated that the salivary cortisol levels of children



with ECC were significantly higher than caries-free children [Pani et al., 2013].

Associations of salivary cortisol levels to psychometric indices have been reported in studies applying other psychological stressors in children, but the samples were limited.

Our results demonstrate that caries presence was positively associated with increasing age and salivary cortisol levels. Moreover, lower and higher levels of plaque and higher levels of tartar were found to be risk factors associated with caries presence. In accordance with previous studies, we found that caries experience was not significantly associated with other factors, such as gender, parent education, diet and oral hygiene, systemic fluoride intake, or birth events [Selwitz et al., 2007; Rai et al., 2010].

## Conclusions

In conclusion, the results of the present study highlight a relationship between salivary cortisol levels and caries experience in children. Dental caries experience was positively associated with increased salivary cortisol levels, plaque, high tartar levels, and increased age. Moreover, increased cortisol levels and increased age were independently associated with caries presence.

Within the limitations of this study, we can conclude that a definite relationships exists between salivary cortisol levels of the child and the development of caries. In future it would be important to understand what are the mechanisms that determine high cortisol levels in children.

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