



OPEN Association between environmental stress factors, salivary cortisol level and dental caries in Egyptian preschool children: a case-control study

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Dental caries remains the most common chronic disease in childhood. This study aimed to identify environmental stressors in children and estimate the association between salivary cortisol level as a stress biomarker and childhood caries. We conducted a case-control study involving 80 Egyptian children. The cases were 40 children with early childhood caries (ECC). The controls included 40 caries-free children. Data were collected from the parents or caregivers of the children using a questionnaire. The clinical examination included caries experience and an oral hygiene index. Saliva samples were collected and tested for unbound cortisol levels. The results from the current study showed that the mean age for participants was 5.09 ± 0.66 years. Twenty-one of the children with ECC were girls. In multivariate regression, there is a significant association between ECC and elevated salivary cortisol levels ($> 0.57 \mu\text{g/dl}$) with an adjusted odds ratio (AOR) of 9.649 ($p = 0.003$), father smoking (AOR = 5.791, $p = 0.030$), sleeping with a bottle or food in the mouth (AOR = 8.481, $p = 0.027$), and poor oral hygiene evaluated through the Silness-Löe Plaque Index (AOR = 6.681, $p = 0.001$). As a conclusion, the study indicates that salivary cortisol is an independent stress biomarker in relation to early childhood caries. Salivary cortisol as a stress biomarker can help clinicians provide an additional assessment tool for identifying caries risk in children.

Keywords Preschool children, Early childhood caries, Environmental risk factors, Salivary cortisol, Stress

Abbreviations

AOR	Adjusted odds ratio
AUC	Area under the curve
CI	Confidence interval
dmft	Decayed, missing, and filled teeth
ECC	Early childhood caries
ESS-CHAD	Epworth sleepiness scale for children and adolescents
MS	Mutans streptococci
OR	Odds ratio
P	p value
ROC	Receiver operating characteristic curve
SD	Standard deviation
U	Mann Whitney test

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WHO

World Health Organization

Dental caries remains the most common chronic disease in childhood. It is five times more common than asthma and seven times more common than hay fever¹. It is considered a multifactorial complex disease that is influenced by a variety of factors, including diet, oral hygiene, socioeconomic status, genetic predisposition, and environmental factors. The interaction of these factors contributes to its development and progression^{2,3}. Global estimates of dental caries prevalence in primary and permanent teeth in 2020 were 46.2% and 53.8%, respectively⁴. The prevalence in developing countries was estimated to be as high as 50–80%⁵. Untreated carious lesions can lead to expensive treatment, disruption of growth and development, pain, and life-threatening infections⁶.

Early childhood caries (ECC) has been defined as the presence of one or more decayed (non-cavitated or cavitated), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger⁷. Early childhood caries is linked to several factors, including infant feeding practices such as prolonged bottle-feeding, especially with sugary liquids; early bacterial exposures, such as *Mutans Streptococci* (MS) often transmitted by caregivers; genetic predisposition that influences susceptibility to caries; environmental risk factors; and socioeconomic conditions, where low socioeconomic status can limit access to dental care and healthy foods, thus increasing the risk of caries. Deciduous teeth are more susceptible to caries than permanent teeth due to several factors including their thinner enamel and dentin that make them more vulnerable to acid attacks, the less organized structure of their enamel that weakens their resistance to decay. Additionally, their higher carbonate content that can compromise their structural integrity. Finally, their shorter development period that can lead to less mineralization and increased caries risk^{8,9}. The direct consequences of ECC are pain and infection. These can affect the child's ability to eat and subsequently reduce growth and weight gain due to insufficient food consumption to meet the metabolic and growth needs of children. Furthermore, its consequences can affect the immediate and long-term quality of life of the child and family^{10,11}.

Previous studies have reported that stress is a significant factor influencing overall health, and has been linked to increased dental caries risk. Increased cortisol is an important biomarker of stress response and has been associated with a higher susceptibility to dental caries¹². Cortisol can induce atrophy of the salivary glands, reducing saliva production (quantity) and altering its composition (quality). These salivary changes may result in increased formation and adherence of cariogenic biofilm, potentially having a detrimental effect on teeth¹³. Furthermore, previous studies have reported that stress hormones such as cortisol can suppress the immune response, inhibiting the activity of essential antimicrobial agents in saliva such as salivary Immunoglobulin A, lactoferrin, lysozyme, and lactoperoxidase, further weakening oral defenses¹⁴.

Additionally, environmental and psychosocial stressors, such as poverty, single-parent households, low parental education levels, parental smoking, and academic challenges, can significantly elevate cortisol levels in children. Such children may eventually develop anxiety, making them prone to dental decay and other medical conditions¹⁵. Agarwal et al. (2023) reported greater prevalence of proximal caries in children with higher anxiety and stress levels in comparison to their counterparts¹⁶. Additionally, unhealthy coping mechanisms, like excessive mobile phone use, can exacerbate oxidative stress, potentially augmenting caries risk¹⁷. This complex interplay of biological, environmental, and psychosocial factors underscores the importance of addressing stress, which plays a substantial role in caries risk in children.

According to a WHO study, the prevalence of dental caries in Egypt is estimated to be 70%¹⁸. A 2019 Egyptian cross-sectional study reported a higher prevalence of dental caries in primary teeth compared to permanent teeth, with 74% of children affected¹⁹. Because of the scarcity of literature on the relationship between environmental factors and salivary cortisol levels in children with dental caries in Egypt, the current study is designed to assess this relationship. This study aimed to identify environmental stressors in a child's everyday life that contribute to dental caries in children and to measure the salivary cortisol level as a stress biomarker for childhood caries.

Methods

Study design and setting

To evaluate the environmental stressors for dental caries in children and to measure the salivary cortisol level as a stress biomarker for childhood caries, a case-control study was conducted among children aged 4 to less than 6 years. The study was conducted at preschool nurseries in Alexandria and at the dental clinic of the Medical Research Institute, Alexandria University, from July 2022 to May 2023.

Sample size and design

Based on the case-control study of Rai et al. (2010), the mean difference between cortisol levels in children with caries and caries-free children was 0.69 µg/dl, and the SD of children with caries was 1.344 µg/dl, SD of caries-free children was 0.337 µg/dl. Using Alpha error = 5% and beta error = 20%, the minimum required sample size was estimated to be 66 children, which was increased to 80, 40 for each group²⁰.

The sample size was calculated using G. Power software. The cluster sample technique was used to select two districts out of ten administrative districts in Alexandria Governorate. From each district, 5 nurseries were chosen randomly, and 4 cases and 4 controls were selected randomly from each nursery.

Study population

Forty children with caries and an equal number of caries-free children matched for age and gender were included. Cases were identified by a pediatric dentist based on clear diagnostic criteria for dental caries. Matched controls with no history of/or current diagnosis of dental caries were then selected from the same pool of population. Cases and controls were selected according to the following criteria:

Inclusion criteria included (1) Child aged 4 to < 6 years; (2) Only one child was chosen from each family.

Exclusion criteria included (1) Children with systemic diseases or special health care needs; (2) Children taking medications that interfere with measures of salivary cortisol.

The data collection tools:

Interview questionnaire

Data on past exposures were collected from the parents or caregivers of the children enrolled in the present study using a pre-structured, pre-coded interview questionnaire. The items of the questionnaire were acquired after meticulous reviewing of the literature and were obtained from previously validated research questionnaires^{21–23}. Additionally, it was assessed by experts in dentistry and public health to be sure of the content validity. The English questionnaire was translated both forward and backward by native speakers who are specialists in public health.

The questionnaire included socio-demographic data comprising parental occupation and education. It also involved potential socio-environmental stressors in the child's everyday life, including socio-economic situation, marital conflict or violence, child abuse or negligence, the death of a family member, and parental smoking. The kindergarten and school environment included being bullied by other children at school, peer pressure, losing games, having difficulties making friends or racism, having learning disabilities, moving to another school, bad grades, and too much homework. Dietary habits and dental hygiene were included in the questionnaire (Supplementary file I). The sleeping pattern of the child was obtained through the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD)²⁴ (Supplementary file II). Medical and dental histories were searched for any significant findings.

Clinical examination

Caries experience The examination was performed using a plane mirror and dental explorer under daylight without drying to assess caries according to WHO criteria. The condition of each surface was recorded as sound, decayed, filled, or missing using the decayed, missing, and filled teeth (dmft) index. The individual dmft value is the sum of the number of decayed, missed due to caries, and filled teeth²⁵. Those without caries (zero score) were considered control participants, and others were recruited in the case group.

Oral hygiene (Silness-Löe plaque Index) The Silness-Löe Plaque Index was used to assess the thickness of plaque at the gingival area of index teeth. The measurement of the state of oral hygiene by the Silness-Löe Plaque Index is based on recording both soft debris and mineralized deposits on the index teeth. The scores from the four areas of the tooth are added and divided by four in order to give the plaque index for tooth²⁶ (Supplementary file III).

Assessment of unbound cortisol level in saliva

Saliva collection To standardize the time of collection, the saliva samples were always collected from children at the same appointment hours. Saliva sampling for each child was scheduled at morning appointments, between 9 a.m. and 12 p.m.²⁷.

Specimen Preparation Eating, drinking, chewing gum, or brushing teeth were avoided for 30 min before saliva sampling. It was recommended to rinse the mouth thoroughly with cold water for 5 min before sampling. Sample collection was postponed if oral diseases, inflammation, or lesions existed that would be a source of blood contamination. If there was visible blood contamination in the patient's saliva specimen, it was discarded. The sampling device was rinsed with water, waited for 10 min, and a new sample was taken.

The collected samples were transferred within a maximum of an hour to the laboratory of the Clinical Pathology Department, Faculty of Medicine, Alexandria University, to be frozen at -20°C and stored until processing time. The average absorbance values for each set of standards, controls, and patient samples were calculated. A calibration curve was constructed, and the results were interpolated from the curve²⁸.

Ethical approval

The study has obtained the approval of the Ethics Committee of the High Institute of Public Health, Alexandria University, Egypt, for conducting the research. The researchers complied with the International Guidelines for Research Ethics. A written informed consent was taken from the caregivers of the children after an explanation of the purpose and benefits of the research. Anonymity and confidentiality were ensured. All methods were performed in accordance with the relevant guidelines and regulations.

Statistical analysis

According to the present research question, lifestyle factors and cortisol level were the exposures, while caries and plaque index were the outcomes. Descriptive statistics were calculated as frequencies and percentages for qualitative variables and means and standard deviations for quantitative variables. Children with ECC and caries-free children were compared regarding sociodemographic factors, oral health practices, dietary habits, and plaque index using the t-test, chi-square, Fisher exact, and Mann-Whitney U tests. Univariate regression analysis investigated the association between environmental stressors, free salivary cortisol (exposures), and the presence of ECC (outcome). Variables with significant associations were entered into a multivariate logistic regression analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the significant risk factors for ECC. The significance level was set at 5%. SPSS version 17.0 was used for statistical analysis.

Results

Socio-demographic characteristics of participants

Table 1 represents the socio-demographic characteristics of both groups. The median age was 5 years old. No statistically significant differences existed between the two groups (children with ECC and caries-free children) as regards age or gender ($p = 0.613$ and 0.655 respectively), indicating homogeneity between the two groups. 55% of the children with ECC were from the East and Middle districts of Alexandria, while most of the caries-free children (67.5%) were from the El Montazah and East districts. No statistically significant differences existed between the two groups as regards the address of the children ($p = 0.627$).

There was no statistically significant difference as regards the order of birth of the child among siblings in both groups ($p = 0.273$). However, there was a statistically significant difference between the two groups as regards the mother's occupation ($p = 0.035$). Most of the mothers of children with ECC were either non-working (47.5%) or had heavy work (40%). On the other hand, 37.5% of the mothers of caries-free children have clerical work. Nearly half of the control group (45%) had highly educated fathers (university or postgraduate education) compared to only 17.5% of the case group, and this difference was statistically significant ($p = 0.037$).

Potential socio-environmental stressors in the child's everyday life

Table 2 demonstrates the comparison between the two studied groups according to potential socio-environmental stressors in the child's everyday life (as stated by the mother or caregiver). The family income was not enough for 37.5% of the families of children with ECC and 25% of the families of caries-free children. However, the difference was not significant ($p = 0.505$).

Socio-demographic characteristics	Cases (n = 40)		Controls (n = 40)		Test of significance	p-value
	N	%	N	%		
Age (years) mean \pm SD	5.09 \pm 0.66		5.01 \pm 0.66		t = 0.507	0.613
Sex						
Male	19	47.5	21	52.5	$\chi^2 = 0.2$	0.655
Female	21	52.5	19	47.5		
Residence						
El Montazah district	8	20.0	10	25.0	$\chi^2 = 5.669$	MC p = 0.627
East district	12	30.0	17	42.5		
Middle district	10	25.0	8	20.0		
Other districts	10	25.0	5	12.5		
Order of birth among siblings						
First	10	25.0	14	35.0	$\chi^2 = 3.995$	MC p = 0.273
Second	16	40.0	14	35.0		
Third	7	17.5	10	25.0		
More	7	17.5	2	5.0		
Maternal education						
Illiterate + Read & write	5	12.5	3	7.5	$\chi^2 = 4.503$	MC p = 0.213
Primary + middle + high	14	35.0	11	27.5		
Above average qualification	11	27.5	7	17.5		
University + Postgraduate	10	25.0	19	47.5		
Mother working						
Non-working	19	47.5	14	35.0	$\chi^2 = 6.684^*$	0.035*
Clerical	5	12.5	15	37.5		
Heavy working	16	40.0	11	27.5		
Paternal education						
Illiterate + Read & write	6	15.0	4	10.0	$\chi^2 = 8.480^*$	0.037*
Primary + middle + high	10	25.0	10	25.0		
Above average qualification	17	42.5	8	20.0		
University + Postgraduate	7	17.5	18	45.0		
Father working						
Non-working	4	10.0	2	5.0	$\chi^2 = 0.761$	MC p = 0.823
Clerical	12	30.0	12	30.0		
Heavy working	24	60.0	26	65.0		

Table 1. Distribution between the two studied groups according to their socio-demographic characteristics (Medical Research Institute, 2023). χ^2 : Chi square test, MC: Monte Carlo, t: t test, p: p value for comparing between the studied groups, *Statistically significant at $p \leq 0.05$.

	Cases (n = 40)		Controls (n = 40)		Test of significance	p-value
	N	%	N	%		
Potential socio-environmental stressors						
Family income						
Enough and exceed	4	10.0	4	10.0	$\chi^2=1.58$	MC p=0.505
Enough	21	52.5	26	65.0		
Not enough	15	37.5	10	25.0		
Parental depression	12	30.0	10	25.0	$\chi^2=0.25$	0.617
Parental separation/ conflict/Divorce	4	10.0	3	7.5	$\chi^2=0.16$	FE p=1.000
Parental violence	7	17.5	3	7.5	$\chi^2=1.83$	0.176
Child abuse or negligence	2	5.0	1	2.5	$\chi^2=0.346$	FE p=1.000
New sibling to the child	13	32.5	14	35.0	$\chi^2=0.06$	0.813
Father smoking	23	57.5	14	35.0	$\chi^2=4.07$	0.044*
Kindergarten and school environment						
Being bullied by other children at school	10	25.0	6	15.0	$\chi^2=1.25$	0.264
Having difficulties making friends or racism	8	20.0	6	15.0	$\chi^2=0.35$	0.556
Having learning disabilities	13	32.5	5	12.5	$\chi^2=4.59$	0.032*
Moving to another school	6	15.0	3	7.5	$\chi^2=1.13$	FE p=0.481
Bad grades	4	10.0	3	7.5	$\chi^2=0.16$	FE p=1.000
Too much homework	11	27.5	8	20.0	$\chi^2=0.62$	0.431
Modified pediatric Epworth sleepiness scale						
Total Epworth score						
Normal (0–10)	22	55.0	34	85.0	$\chi^2=9.77$	MC p=0.012*
Mild (11–14)	12	30.0	3	7.5		
Moderate (15–17)	4	10.0	3	7.5		
Severe (18–24)	2	5.0	0	0.0		
Min. – Max.	0.0–18.0		0.0–16.0		U=518.50	0.007*
Mean ± SD.	9.10 ± 5.15		6.08 ± 4.47			
Median (IQR)	10.0 (4.50 – 12.50)		6.0 (2.0–9.0)			

Table 2. Comparison between the two studied groups according to potential socio-environmental stressors in the child's everyday life (Medical Research Institute, 2023). χ^2 : Chi square test, MC: Monte Carlo, FE: Fisher Exact, U: Mann Whitney test, p: p value for comparing between the studied groups, *: Statistically significant at $p \leq 0.05$.

There was no significant difference concerning the incidence of parental depression (30% in cases vs. 25% in controls) ($p = 0.617$) or parental separation (10% in cases vs. 7.5% in controls) ($p = 1.00$). Although parental violence was reported by 17.5% of children with ECC compared to 7.5% of caries free children, the difference did not reach a statistically significant level ($p = 0.176$).

Having learning disabilities was reported by 32.5% of children with ECC, compared to 12.5% of caries-free children. There is a statistically significant difference between the two groups as regards having learning disabilities ($p = 0.032$).

On the other hand, there was no statistically significant difference between the two groups in relation to other factors, including being bullied by other children at school (25% in cases, 15% in controls, $p = 0.264$), having difficulties making friends or racism (20% in cases, 15% in control, $p = 0.556$), moving to another school (15% in cases, 7.5% in controls, $p = 0.481$), bad grades (10% in cases, 7.5% in controls, $p = 1.000$) and too much homework (27% in cases, 20% in controls, $p = 0.431$). Most fathers of children with ECC (57.5%) were smokers, while only 35% of the fathers of caries-free children were smokers. The difference was statistically significant ($p = 0.044$).

Table 2 also shows the comparison between the two groups in relation to the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD). There were mild sleeping problems in 30% of children with ECC and in only 7.5% of caries-free children ($p = 0.012$), while moderate sleepiness was detected in 10% of children with ECC and in only 7.5% of caries-free children. The mean ESS-CHAD in children with ECC was significantly higher (9.10 ± 5.15) than that of caries-free children (6.08 ± 4.47) ($p = 0.007$).

A comparison between the two groups in relation to dental history and dietary habits is shown in Table 3. Exclusive breast feeding was reported by 72.5% of children with ECC, compared to 75% of caries-free children. The difference was not statistically significant ($p = 0.393$). Sleeping with a bottle or food in the mouth was reported

Dental history & dietary habits	Cases (n = 40)		Control (n = 40)		χ^2	p-value
	N	%	N	%		
Previous dental visits						
No	19	47.5	27	67.5	3.274	0.070
Yes	21	52.5	13	32.5		
Infant milk feeding habit						
Breast feeding	29	72.5	30	75.0	1.937	MC _p = 0.393
Bottle feeding	7	17.5	9	22.5		
Mixed	4	10.0	1	2.5		
Sleeping with bottle /food in the mouth						
No	30	75.0	37	92.5	4.501*	0.034*
Yes	10	25.0	3	7.5		
Wake up to eat /drink juice						
No	13	32.5	19	47.5	1.875	0.171
Yes	27	67.5	21	52.5		
Cleaning teeth using toothbrush						
No	7	17.5	7	17.5	0.096	0.953
Once daily	26	65.0	27	67.5		
Twice daily	7	17.5	6	15.0		
Brushing teeth before bedtime	(n = 33)		(n = 33)			
No	14	42.4	8	24.2	2.455	0.117
Yes	19	57.6	25	75.8		
Using fluoride toothpaste						
No	15	37.5	17	42.5	0.208	0.648
Yes	25	62.5	23	57.5		
Parental supervision on teeth brushing						
No	13	32.5	15	37.5	0.220	0.639
Yes	27	67.5	25	62.5		

Table 3. Comparison between the two studied groups according to dental history and dietary habits (Medical Research Institute, 2023). χ^2 : Chi square test, MC: Monte Carlo, p: p value for comparing between the studied groups, *Statistically significant at $p \leq 0.05$.

by 25% of children with ECC compared to only 7.5% of caries-free children; the difference was statistically significant ($p = 0.034$).

Tooth brushing was reported by 82.5% of children with ECC and 82.5% of caries-free children ($p = 0.953$). Brushing teeth before bedtime was done by 57.6% of children with ECC and 75.8% of caries-free children ($p = 0.117$). Children use fluoridated toothpaste in 62.5% of cases and 57.5% in the controls group ($p = 0.648$). Parental supervision during tooth brushing was reported by 67.5% of children with ECC and 62.5% of caries-free children ($p = 0.639$).

Clinical examination of the children and salivary cortisol levels

Table 4 shows the caries experience of children with ECC. The dmft index range was 1.0–20.0, with a mean \pm SD of 6.83 ± 4.56 . Table 4 also shows the Silness-Löe Plaque Index of children with ECC and caries-free children. The mean Plaque Index in children with ECC was significantly higher (1.28 ± 0.51) than that of caries-free children (0.34 ± 0.37) ($p < 0.001$). The mean salivary cortisol level in children with ECC (0.67 ± 0.23 $\mu\text{g/dl}$) is significantly higher than that of caries-free children (0.50 ± 0.22 $\mu\text{g/dl}$) ($p = 0.003$).

Receiver operating characteristic curve (ROC curve) for salivary cortisol level to predict cases

The ROC curve was plotted to define the cut-off point for salivary cortisol level (Fig. 1). The Area Under the Curve was large enough (AUC) = 0.696; 95% CI = 0.571–0.820, $p = 0.003$ that salivary cortisol level can be considered a predictor of early childhood caries with a sensitivity of 80% and a specificity of 72.5%. The optimal cut-off point was found to be > 0.57 $\mu\text{g/dl}$ (Table 5).

80% of children with ECC had a salivary cortisol level > 0.57 $\mu\text{g/dl}$, which was more than double the percentage of caries-free children in the control group (27.5%) that had a salivary cortisol level > 0.57 $\mu\text{g/dl}$. The difference was statistically significant ($p < 0.001$). Concerning the risk of caries, children with a salivary cortisol level > 0.57 $\mu\text{g/dl}$ revealed a significant risk for caries (Odds Ratio (OR) = 10.545; 95% confidence interval (CI): 3.73–29.84) compared to those with salivary cortisol ≤ 0.57 $\mu\text{g/dl}$ (Table 6).

Clinical examination	Cases (n = 40)	Control (n = 40)	U	p-value
dmft index				
Min. – Max	1.0–20.0	0.0–0.0	0.001	<0.001*
Mean ± SD	6.83 ± 4.56	0.0 ± 0.0		
Median (IQR)	6.0 (3.0–10.0)	0.0 (–)		
Silness and Loe plaque index				
Min. – Max.	0.11–2.0	0.0–1.75	123.50	<0.001*
Mean ± SD	1.28 ± 0.51	0.34 ± 0.37		
Median (IQR)	1.33 (1.0–1.75)	0.25 (0.04–0.52)		
Salivary cortisol level µg/dl				
Min. – Max.	0.16–0.99	0.26–0.90	487.0	0.003*
Mean ± SD.	0.67 ± 0.23	0.50 ± 0.22		
Median (IQR)	0.73 (0.60–0.81)	0.41 (0.33–0.65)		

Table 4. Comparison between the two studied groups according to clinical examination of the child and salivary cortisol level (Medical Research Institute, 2023). U: Mann Whitney test, p: p value for comparing between the studied groups, *Statistically significant at $p \leq 0.05$, SD: Standard deviation, IQR: Interquartile range.

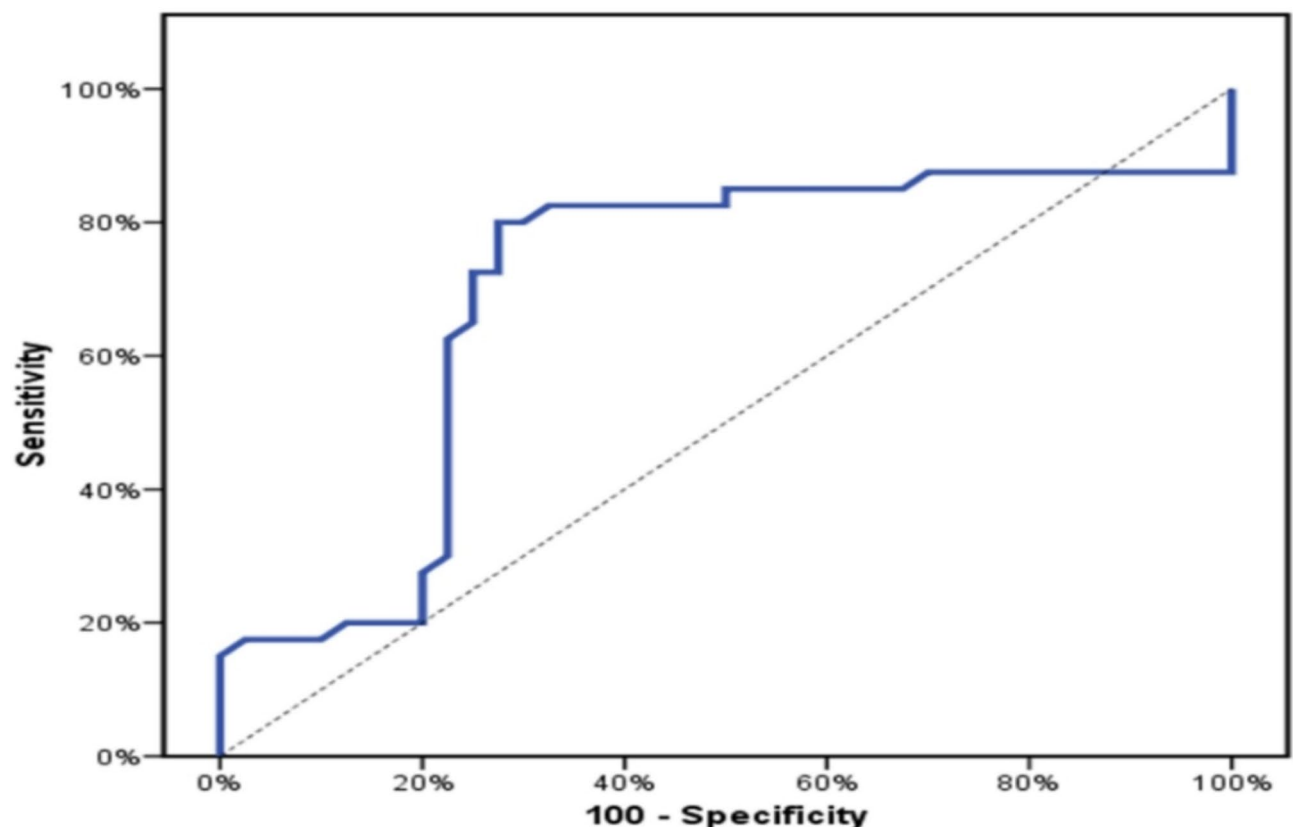


Fig. 1. ROC curve for salivary cortisol level to predict cases.

Estimation of the association between caries in children and environmental stressors

Table 7 shows the results of the logistic regression analysis investigating the association between ECC and study variables: maternal education, paternal education, father smoking, having learning disabilities, total Epworth score, sleeping with a bottle, Silness-Löe Plaque Index, and salivary cortisol level > 0.57 µg/dl.

In univariate regression, a significant association was observed with father smoking, having learning disabilities, total Epworth score, sleeping with a bottle or food in the mouth, Silness-Löe Plaque Index, and salivary cortisol level (> 0.57 µg/dl). Silness-Löe Plaque Index, and salivary cortisol level (> 0.57 µg/dl) were associated with higher odds of ECC (OR = 8.671 and 10.545).

	AUC	P-value	95% CI		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Salivary cortisol level	0.696*	0.003*	0.571	0.820	> 0.57	80.0	72.5	74.4	78.4

Table 5. Agreement (sensitivity, specificity) for salivary cortisol level to predict cases (Medical Research Institute, 2023). AUC: Area under a Curve, P value: Probability value, CI: Confidence Intervals, *Statistically significant at $p \leq 0.05$, PPV: Positive predictive value, NPV: Negative predictive value.

Salivary cortisol level	Cases (n = 40)		Control* (n = 40)		OR	95%CI	p-value
	No.	%	No.	%			
≤ 0.57 µg/dl (-ve) *	8	20.0	29	72.5			
> 0.57 µg/dl (+ ve)	32	80.0	11	27.5	10.545	3.73–29.840	< 0.001*

Table 6. Comparison between the two studied groups according to salivary cortisol cut off level (Medical Research Institute, 2023). OR Odds ratio, CI Confidence interval, LL Lower limit, UL Upper Limit, *: Reference group.

	Univariate		Multivariate*	
	p-value	OR (95% CI)	p-value	OR (95% CI)
Mother working (non-working)	0.258	0.595 (0.242–1.462)		
Paternal education	0.644	1.238 (0.500–3.066)		
Father smoking	0.045*	2.513 (1.019–6.198)	0.030*	5.791 (1.183–28.336)
Having learning disabilities	0.038*	3.370 (1.070–10.613)	0.334	2.378 (0.410–13.782)
Total Epworth score	0.009*	1.138 (1.033–1.255)	0.528	1.056 (0.891–1.253)
Sleeping with bottle /food in the mouth	0.044*	4.111 (1.037–16.295)	0.027*	8.481 (1.272–56.554)
Silness-Löe Plaque Index	< 0.001*	8.671 (3.391–22.169)	0.001*	6.681 (2.239–19.931)
Salivary cortisol level (> 0.57 µg/dl)	< 0.001*	10.545 (3.73–29.840)	0.003*	9.649 (2.199–42.343)

Table 7. Univariate and multivariate logistic regression analysis for the parameters affecting dental caries (Medical Research Institute, 2023). OR Odds ratio, CI Confidence interval, LL Lower limit, UL Upper Limit, # All variables with $p < 0.05$ was included in the multivariate, *Statistically significant at $p \leq 0.05$.

All significant variables ($p < 0.05$) in the univariate analysis (father smoking, having learning disabilities, total Epworth score, sleeping with a bottle/food in the mouth, Silness-Löe Plaque Index, and elevated salivary cortisol) were included in a multivariate logistic regression model. This model revealed that only four factors were significantly associated with an increased risk of early childhood caries (ECC). The strongest predictor of ECC was elevated salivary cortisol levels (> 0.57 µg/dl) with an adjusted odds ratio (AOR) of 9.649 ($p = 0.003$). Other significant factors were father smoking (AOR = 5.791, $p = 0.030$), sleeping with a bottle or food in the mouth (AOR = 8.481, $p = 0.027$), and poor oral hygiene evaluated through the Silness-Löe Plaque Index (AOR = 6.681, $p = 0.001$).

Discussion

Early childhood caries is a multifactorial disease and has numerous biological, psychological, behavioral, and socio-environmental risk factors²⁹. Salivary cortisol has been used as a biomarker to explore the role of stress in children with ECC. Therefore, the goal of this study was to assess the salivary cortisol level as a stress biomarker in relation to environmental risk factors for ECC.

The present study revealed that the mean salivary cortisol level in children with ECC (0.67 ± 0.23 µg/dl) is significantly higher than that of caries-free children (0.50 ± 0.22 µg/dl) ($p = 0.003$). There is a significant association between free salivary cortisol and ECC at both univariate and multivariate logistic regression analysis. These results indicate that salivary cortisol is an independent stress biomarker in relation to early childhood caries. This is in accordance with another case-control study that stated that high salivary cortisol level was an independent factor associated with early childhood caries, with OR values of 3.05 (95% CI: 1.84–5.06) and 1.59 (95% CI: 1.09–2.58)³⁰. There are also several studies that stated that salivary cortisol can be used as a tool to measure child's stress in relation to ECC^{12,20,31,32}.

According to the findings of our study, the statistically significant difference in parental education levels between cases and controls suggests that lower socioeconomic status, often indicated by lower education, may be a risk factor for childhood dental caries. This is similar to an Egyptian cross-sectional study that concluded that low parental education may be a risk factor for dental caries¹⁹. A Romanian study stated that the low level

of education of parents was related to the high risk of caries among school children. The study concluded that parental education is reflected in the dental knowledge, behavior, and general care of the child and consequently contributes to the caries risk³³.

Parents with lower education levels may have limited knowledge about oral health, leading to poor oral hygiene practices and dietary habits among their children. Additionally, socioeconomic challenges and increased stress experienced by these parents can indirectly impact their children's oral health by elevating cortisol levels and compromising the oral defenses^{15,34}.

Our data showed a statistically significant association between paternal smoking and a higher risk of dental caries in children. Children of smoking fathers were 2.51 times more likely to develop dental caries compared to children of non-smoking fathers ($p=0.046$, 95% CI = 1.019–6.198). That is in accordance with other studies suggesting paternal smoking as a risk factor for ECC. Exposure to secondhand smoke can negatively impact a child's oral health. While the exact mechanisms aren't fully understood, it's believed that secondhand smoke can reduce salivary flow, which has a vital role in washing away food particles and bacteria, increasing harmful bacteria, or impairing the immune system in the affected child^{35–37}. Besides, exposure to secondhand smoke can lead to increased stress and elevated cortisol levels in children^{38,39}.

Children with dental caries were more likely to have learning disabilities ($p=0.032$) as shown in the present study. This was supported by a cross-sectional study that estimated the prevalence of dental caries in children with disabilities to be high⁴⁰. On the other hand, a meta-analysis by Robertson et al. (2019) did not find a significant difference in caries levels between children with and without learning disabilities⁴¹. Some disabilities may make it challenging for children to understand the importance of oral hygiene or follow a consistent routine. Besides, children with certain disabilities might be sensitive to the taste of toothpaste, the feeling of a toothbrush, or the sound of dental equipment, making brushing a struggle. In addition, certain disabilities may be linked to weakened immune systems, making children more susceptible to oral infections that contribute to ECC⁴⁰.

Children with learning disabilities often experience increased stress and elevated cortisol levels due to academic challenges, social difficulties, and the pressure to keep up with their peers. Chronic stress can negatively impact oral health by suppressing the immune system, altering salivary composition, and hindering the body's natural repair processes. These factors can create an environment conducive to the growth of cariogenic bacteria, leading to increased susceptibility to dental caries^{12,42}.

The present study revealed a significant difference in the quality of sleep between cases and controls, as measured by the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD). To our knowledge, this is the first study to investigate the relationship between the ESS-CHAD and dental caries in children. A study stated that the risk of early childhood caries could correlate to sleep disturbances⁴³, another study suggested that sleep problems among young children were a behavioral risk factor for night-time bottle use and early childhood caries⁴⁴. In a Japanese population based cohort study, the Odds Ratios for children with short or irregular sleep duration compared with those with sleep duration of ≥ 11 h were 1.30, 1.16, 1.11, and 1.35 for sleep duration of ≤ 8 , 9, 10 h, and irregular sleep duration, respectively, concluding that late bedtime and short sleep duration were both consistently associated with increased risk of caries in deciduous teeth⁴⁵. Insufficient sleep can lead to elevated levels of stress hormones like cortisol which subsequently increase the risk of dental caries by promoting the growth of harmful bacteria, reducing saliva protective effects, and hindering the remineralization of tooth enamel^{46,47}.

In our study, sleeping with bottle or food in the mouth was reported by 25% of children with ECC compared to only 7.5% of caries free children, the difference was statistically significant ($p=0.034$). Children sleeping with bottle or food in their mouth had 4.11 times more risk to develop caries than those children did not have this habit ($p=0.044$, 95% CI = 1.037–16.295). Sleeping with a bottle or food in the mouth significantly increases ECC because sugary liquids pool around teeth for extended periods, reducing saliva flow (which naturally cleans teeth) and allowing cavity-causing bacteria to thrive^{48,49}.

The current study demonstrated that the plaque index is a risk factor for the development of caries (OR = 52.20, 95% CI = 10.63–256.34). This is in agreement with the results of a study conducted by Caruso et al. (2018), that 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 levels³⁰.

The relationship between stress and oral health is bidirectional. Stress can negatively impact oral health by elevating cortisol levels, which can suppress the immune system, alter salivary composition, and hinder the body's natural repair processes. This can create an environment conducive to the growth of cariogenic bacteria, leading to increased susceptibility to dental caries and periodontal disease. Conversely, oral health problems, such as dental pain or periodontal disease, can exacerbate stress and anxiety, creating a vicious cycle³². Coxon et al. (2019) investigated the relationship between dental anxiety and oral health in children aged 5 and 8 years. The study found a strong association between dental anxiety and poorer oral health outcomes, including increased decay experience, active decay, and the need for dental restorations⁵⁰. Although some studies have explored the relationship between dental caries, stress, and cortisol levels, further research is needed to solidify this connection. More studies are required to measure cortisol levels early before the development of dental caries and to monitor these levels throughout the disease progression and treatment process. This would provide a clearer understanding of the causal relationship between stress, cortisol, and the risk of dental caries in children.

Limitations and strengths of the study

The present study has some limitations. First, in case-control studies, the possibility of recall bias can't be avoided completely since the majority of exposure data was derived from self-reported history. The study's design also made it more difficult to account for all possible confounders and assign causal links to the results that were discovered. Second, the possible impact of genetic heterogeneity on the relationship between risk factors and dental caries could not be ascertained due to the absence of genetic information for the studied participants.

Third, the small sample size may make it challenging to ascertain whether a given result is a trustworthy finding. Further research with larger sample sizes is needed to confirm our results. Additionally, long-term follow-up studies are necessary to evaluate the long-term impact of early childhood stress on oral health outcomes. On the other hand, this study has many points of strength. First, the present research studied the environmental stressors of ECC, which were seldom studied in Egypt. In addition, it measured the salivary cortisol level, which is considered an objective indicator of stress that could affect the oral health of children. Besides, it calculated the cut-off level of salivary cortisol; above it, the risk for ECC increased significantly. We used various validated tools to obtain scientifically sound results and to overcome the study limitations.

Conclusion & recommendations

Based on the results of the current case-control study, we can conclude that the salivary cortisol level as a stress biomarker may be a significant risk factor associated with the development of early childhood caries. The significant association between the salivary cortisol level and ECC at both univariate and multivariate logistic regression analysis indicates that salivary cortisol is an independent stress biomarker in relation to ECC.

The mean pediatric Epworth sleepiness score in children with ECC was significantly higher than that of caries-free children, shedding light on the role of sleep disturbance in enhancing ECC. Parental education is reflected in the dental knowledge, behavior, and general care of the child and consequently contributes to caries risk. Paternal smoking, children with learning disabilities, children sleeping with a bottle or food in their mouth, or a high dental plaque index increase the risk of ECC.

In light of the results of the present study, it is recommended that pediatric dentists and public health specialists should collaborate to establish education sessions for family members and caregivers in the kindergartens, schools, and clubs to raise their knowledge and awareness about environmental stress factors and their relation to ECC and how to reduce them. They should also help teachers recognize psychological health problems among the children whom they teach to enable timely detection of stress factors at school and the initiation of the necessary protective measures.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 5 May 2024; Accepted: 13 March 2025

Published online: 01 April 2025

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Acknowledgements

The research team expressed their sincere gratitude to the volunteers and their caregivers who kindly offered their time to participate in the study.

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Conceptualization, M.F.H., and Y.A.H.; Methodology, M.F.H. and M.H.A.; Software, Y.A.H. and M.F.H.; Formal Analysis, Y.A.H. and M.F.H.; Data curation: Y.A.H., and M.F.H.; Resources, Y.A.H. and M.E.B.; Writing–Original Draft Preparation, M.F.H., R.H.R., and M.H.; Writing–Review & Editing, M.F.H., M.H., and R.H.R.; selection of cases and controls, Y.A.H., and O.M.E.; Visualization, Y.A.H.; Supervision, M.H.A., and M.F.H. All

authors have read and agreed to the published version of the manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the High Institute of Public Health, Alexandria University, Egypt.

Consent for publication

The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration (from you or one of your Contributing Authors) by another publisher. All of the material is owned by the authors and/or no permissions are required.

Informed consent

Before the start of the research, each participant's caregiver provided their informed permission.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-94327-0>.

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