REVIEW ARTICLE

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Non-Invasive Measurement of Cortisol in Children with Adverse Childhood Experiences: A Systematic Review

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Keywords

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Abstract

Purpose

Adverse Childhood Experiences (ACEs) encompass various traumatic events during childhood, linked to adverse health outcomes in later life. Traditional screening methods have possible pitfalls, leading to a growing interest in the use of biomarkers.

Methods

A comprehensive literature review was conducted resulting in the inclusion of 27 studies examining biomarkers of toxic stress in children. These studies employed diverse methodologies, including hair and saliva sampling techniques, and utilized various assessment tools for childhood adversity.

Results

The studies analyzed 1,787 hair samples and 4,170 saliva samples for associations between childhood adversity and cortisol. Hair cortisol concentration (HCC) showed a robust positive correlation with cumulative adversity, while salivary cortisol measures showed a less consistent correlation to adversity.

Discussion

Age, gender and adversity influence the hypothalamic pituitary adrenal (HPA) axis. Hair cortisol emerged as a possible biomarker for chronic stress exposure, while salivary cortisol measures offered insights into acute stress responses with conflicting results. The discrepancies in findings highlight the need for further research to elucidate the complex interplay between childhood adversity and cortisol dynamics, informing targeted interventions to mitigate long-term health effects.

Introduction

Adverse Childhood Experiences (ACEs) encompass traumatic events and chronic stressors occurring during childhood, including physical, emotional, or sexual abuse, parental neglect, household dysfunction, and exposure to violence or substance abuse (1–3). The first ACE study was published in 1998 and since then research has extensively documented the profound impact of ACEs on physical, emotional, and cognitive development, correlating with an increased risk of physical and psychological morbidity, and premature mortality in early adulthood (4–6). Effectively identifying and mitigating these adversities, particularly in paediatric healthcare, remains a complex challenge due to their multifactorial nature and the limitations of current screening approaches (4–7). Traditional screening methods, often reliant on self-reporting or clinical interviews, are prone to recall and reporting bias, stigma, and subjective interpretation (8,9). Additionally, the potentially invasive nature of some assessments may retraumatize vulnerable children and undermine the therapeutic relationship between healthcare providers and patients (10). In light of these challenges, there is growing interest in biomarkers, such as hair and salivary cortisol, as non-invasive screening tools for ACEs in children (8,11). Cortisol, a stress hormone, can be measured in hair and saliva to offer objective insights into individuals' long-term and acute stress experiences (2,3,12). These biomarkers present advantages, including potential for objective measurement and ability to capture chronic and acute stress responses over time (13). Biomarkers such as hair and salivary cortisol are investigated as non-invasive tools for screening ACEs. Cortisol measurement in hair and saliva

TABLE 1: Development of search query

Designated number	Search query component	explanation
#1	"Adverse childhood experiences" OR "ACEs" OR "Childhood trauma" OR "childhood adversity"	This part of the search query includes multiple synonymous terms related to adverse childhood experiences (ACEs) or childhood trauma.
#2	"biomarkers" OR "Cortisol" OR "Hair cortisol" OR "salivary cortisol" OR "stress biomarkers" OR "Cortisol levels" OR "non-invasive screening methods"	the search is broadened to include various terms related to biomarkers and cortisol, which are indicators of biological processes, particularly stress. Including terms like "Hair cortisol," "salivary cortisol," and "stress biomarkers" ensures coverage of specific types of biomarkers relevant to the study of childhood trauma.
#3	"Children" OR "paediatric population" OR "paediatric screening"	This part of the search query specifies the population of interest, focusing on articles related to children or the paediatric population. It ensures that articles discussing biomarkers and childhood trauma are relevant to the paediatric context.
#1 AND #2 AND #3	("Adverse childhood experiences" OR "ACEs" OR "Childhood trauma" OR "childhood adversity") AND ("biomarkers" OR "Cortisol" OR "Hair cortisol" OR "salivary cortisol" OR "stress biomarkers" OR "Cortisol levels" OR "non-invasive screening methods") AND ("Children" OR "paediatric population" OR "paediatric screening")	

correlate with long-term and acute stress, offering advantages in capturing objective measurement of chronic and acute responses. Advancing knowledge of these biomarkers, alongside consideration of demographic and psychological variables (1), may enable early detection and intervention strategies for ACEs, reducing the long-term sequelae of childhood trauma. This review aims to explore the use of hair and salivary cortisol as biomarkers for ACEs in children, examining their efficacy, feasibility, and ethical considerations within paediatric care.

Methods

Search strategy

A systematic literature review was conducted between June and September 2024. Based on a scoping search, keywords and synonyms were identified for three domains: non-invasive cortisol measurement, adverse childhood experiences, and childhood context (Table 1). The population was defined as individuals younger than 18 years, thereby including infants, children, and adolescents irrespective of whether specific age-related keywords were explicitly included in the search terms. Searches were run in PubMed, Embase, and Web of Science, with queries iteratively refined for relevance (Table 2). Controlled vocabulary terms (e.g., MeSH) were not included in the search strategy.
Based on the scoping phase, the final search strategy yielded studies across a broad range of ages and contexts and was considered sufficiently comprehensive for this systematic review.

Study selection

The search was conducted between June 2024 and September 2024 and identified 363 articles. After removing duplicates, titles and abstracts were screened against predefined criteria by two independent reviewers (MD, LH), with disagreements resolved by a third (ADG). Studies were eligible for full-text review if they included participants younger than 18 years; employed case-control, cross-sectional, longitudinal, or randomized controlled design; were published in peer-reviewed journals, and available in English. Exclusion criteria included non-human studies; case reports, reviews, or meta-analyses; interventions involving medication or treatment; absence of ACE reporting; or lack of adjustment for confounders. Sixty-three studies met initial criteria, but eleven lacked full text availability. Ultimately, 27 articles were included (PRISMA diagram, Fig. 1). Abstract and full-text screening were conducted using Rayyan software (14). Before submission in October 2025, the search was repeated and no new articles were included.

Data extraction

MD extracted data on the characteristics of the report, the study population and outcome parameters. These data were verified for accuracy and completeness by LH and ADG. A risk of bias assessment was using the Newcastle-Ottawa Scale for observational studies (15).

Protocol

Prior to initiation of the review process, the protocol for this study was published on PROSPERO (CRD42024571586).

Results

Study characteristics

From the initial yield of the search strategy, 300 studies were excluded, primarily due to the wrong population (n=144), wrong outcome (n=96), inappropriate study design (n=36) or the absence of reported ACEs (n=36). The assessed risk of bias in the selected studies was low overall. However, some studies selectively sampled participants from hospital settings or via social services, potentially introducing selection bias. Consequently, the study population may not fully represent broader community samples. Scores are summarized in Table 3.

Demography of study participants

The demographic characteristics of study participants are summarized in Table 4. The 27 studies included in this review encompass a total of 1787 assessments of hair and 4170 assessments of saliva samples. Of hair samples, 36.8% (N=657) were from boys, 49.5% (N=884) from girls, and 13.8% (N=246) were not specified. The gender difference may be explained by insufficient hair availability for sampling or refusal for cosmetic reasons. Saliva originated from 35.7% boys (N=1488) and 38.5% girls (N=1606) (25.8% unspecified; N=1076). Gender was not reported in a study where participants were infants (1) and by several studies where gender of the participants was specified for the whole study population, but not for the population whose test samples were eligible for analysis (11,16–18). Participants from a wide range of age groups were included, from infants (youngest group mean age 12.4 months) (1), over toddlers (2,19–21) and school children (13,22–28) to adolescents(11,16,17,22–24,26,27,29–35) (eldest group aged 12-21 years old).

Assessment of adverse childhood experiences

Heterogeneity existed in the methodology for assessment of childhood adversity. Some studies developed their own questionnaire and divided adversities into multiple categories (1,2,19,20,24,25,32). Other authors used established instruments, including the Childhood experiences of Care and Abuse interview (CECA) (17,30,33,36), the Early Life Stress Questionnaire (ELSQ) (35), the Trauma Symptom Checklist for Children (TSCC) (34), the Childhood Trauma Questionnaire (CTQ) (31,37–39), the Lifetime Incidence of Traumatic Events Parent-report (LITE-PR) (13), the Early Trauma Inventory (ETI) (22,26) and the Life Experiences Survey (21). Any categories of adversity reported further in this review are taken from the original research. Few studies considered the temporal relation between the onset of adversities and cortisol measurements. Bosch et al. questioned participants across developmental stages and reported differences in cortisol profiles by timing of adversity (32). Do Prado et al. separately questioned stress levels over the past 12 months but observed no case-control differences (39). Some authors report timing of adversity as a variable in their questionnaires, without integrating this into analyses (11,21,28,35). None of the remaining articles reported timing of adversity.

Non-invasive measurement of cortisol

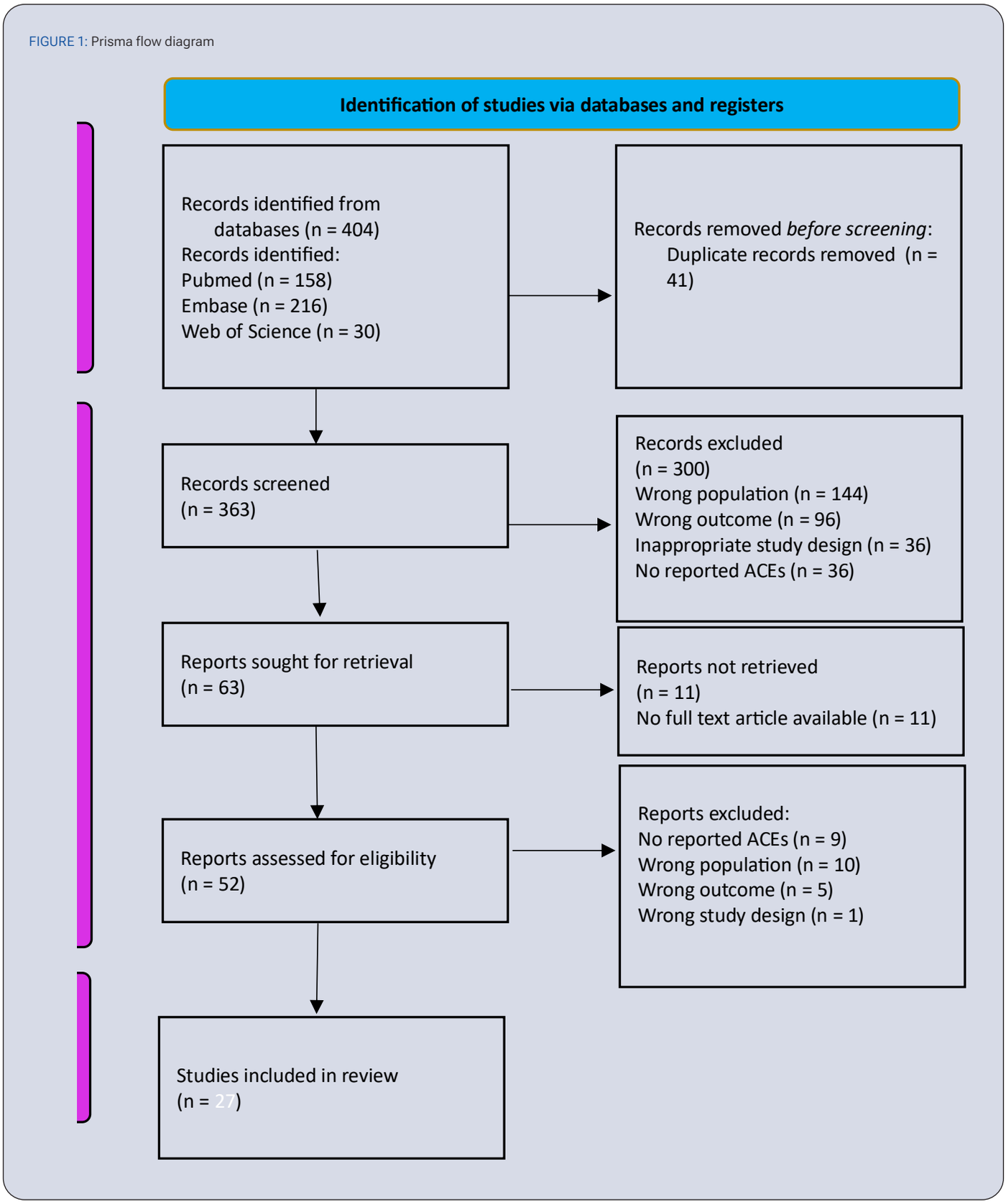
Hair cortisol

Nine studies measured hair cortisol concentration (HCC) (1,2,11,17,19,20,26,30,39), comprising 1787 samples and 35.0% of all cortisol measurement. Sampling was generally uniform with hair collected from the posterior vertex and analysis of the proximal 3 cm. Bryson et al. sampled from the parietal or temporal regions if posterior vertex samples were insufficient (2,20). Van Dammen et al. analysed only the first cm of hair due to a higher number of short hair samples (11). Multiple studies reported positive correlations between ACEs and HCC, of which two in the context of psychiatric comorbidity (17,30), discussed in section 3.4.3. Bhopal et al. reported a strong association between cumulative adversity and hair cortisol in infants. The mean HCC was 6.26 pg/mg (SD 3.01). Each additional adversity corresponded to a 6.1% increase in cortisol levels (95% CI 2.8 – 9.4, p<0.001). The authors structured ACEs in multiple categories, of which HCC changes were most pronounced in relationships, child, and socioeconomic adversities (1). Bryson et al. linked unsafe living conditions to elevated cortisol levels in 2- and 3-year-olds. Mean HCC was 1.04 pg/mg (SD 0.97) for children living in a safe place versus 1.63 pg/mg (SD 0.96) for those who did not. This finding remained significant after adjustment for confounders (β=0.57, 95% CI=0.08–1.07, p=0.023), however without significant correlation to other adversities. Adverse experiences were associated with externalizing symptoms (b=0.41; 95% CI 0.15 to 0.66; no absolute numbers reported), but hair cortisol was not considered a mediating factor (2,20) In a small cohort, Simmons et al. reported significantly higher mean HCC in males than females (5.78 SD 3.88 vs 2.95 pg/mg SD 3.64) and found lifetime trauma positively associated with HCC. Greater trauma exposure, both in frequency and type, predicted elevated HCC (β=0.23, CI 0.01-0.36, p=0.04)(13). A follow-up study confirmed this association and further showed that positive parental behaviours, including nurturing behaviour (b=−0.28, 95% CI -0.50 to -0.06, p=0.014) and maternal emotional coaching (b=−0.25, 95% CI -0.48 to -0.03, p=0.028), correlated with lower HCC (19). In the research of Van Dammen et al. on the contrary, median HCC was significantly higher in female than male participants, respectively 3.6 pg/mg vs 2.4 pg/mg. Total adversity scores were not significantly associated with hair cortisol levels in adolescents. In this population only boys exposed to person-related adversity exhibited statistically significant higher levels of hair cortisol (B=0.016, p=0.007) (11). Hartmann do Prado et al. found that adolescents experiencing early life stress as assessed by the Childhood Trauma Questionnaire had significantly higher HCC (x^2=8.58, p=0.003) (39). Taken together, most studies suggest a positive association between cumulative adversity and hair cortisol concentrations, particularly in younger populations. However, substantial heterogeneity exists in absolute values, effect sizes, and sex-specific findings, limiting comparability across studies.

TABLE 2: Database search strategy

Search query number	database	Search query component	results	Results imported into EndNote
1	PubMed	#1 AND #2 AND #3	150	150
2	Embase	#1 AND #2 AND #3	915	0
3	Embase	'Childhood adversity' AND ('salivary cortisol' OR 'hair cortisol') AND 'child'	74	74
7	Web of Science	#1 AND #2 AND #3	139.072	0
8	Web of Science	childhood adversity hair cortisol salivary cortisol child (ALL FIELDS)	29	29

FIGURE 1: Prisma flow diagram



Salivary cortisol

Diurnal pattern analysis

Fourteen studies measured the diurnal pattern of salivary cortisol (1,11,17,18,22,23,27,28,30,31,34–36,40), reporting data for 1977 participants (33.2% of cortisol measurement). Timing of sampling differed among study groups. The timing of cortisol sampling is summarized in Table 5. Five studies were conducted in het context of psychiatric comorbidity, discussed in section 3.43. (17,27,30,31,35). Valentino et al. saw significantly lower morning cortisol levels in maltreated children aged 3-6 years old, compared to controls (mean 0.32 mg/mL (SD=0.32) vs 0.48 mg/mL (SD=0.52), SE=0.16, p=0.04). Midday and evening cortisol

showed no significant difference (40). Marques-Feixa et al. identified blunted decline of salivary cortisol during the day and thus a higher cortisol area-under-the-curve (AUC) in maltreated children and adolescents, when analysed in an ANOVA test. This result showed dose-response effects for severity (F=6.349, p=0.002) and frequency (F=6.068, p=0.003) of maltreatment. When differentiating age groups, cortisol levels were higher in adolescents compared to younger children (18). Kuhlman et al found trauma-specific differences in a cohort of 9 to 16-year-olds, with non-intentional trauma linked to a steeper diurnal slope (non-intentional × Time b=−.037, t (103.3) =−2.36, p=02). Age and sex were not found to be significant predictors of the cortisol awakening response (22). Kaess et al. observed an elevated cortisol awakening response only in girls with higher Childhood Trauma Questionnaire (CTQ) scores (B=-0.652/β=-0.026, p=0.047) (37). In 15 participants living in residential care homes for abused adolescents, mean cortisol AUC correlated with internalizing symptoms (r=0.56, p<0.05) (34). Several studies report trends in results without reaching statistical significance. These results must be interpreted with caution. Bhopal et al. reported correlation between adversity and a blunted diurnal cortisol slope in infants with small effect sizes. The overall mean saliva AUC in their cohort was 1.29 mcg/dL (SD 0.47), with 60% showing a cortisol slope smaller than 0.01 mcg/h and 15.3% showing a raising slope instead of the hypothesized decline (1). No significant relation between fetal or childhood psychosocial adversity and cortisol could be demonstrated by Isaksson et al. in children and adolescents with ADHD, values were not reported (27). Van Dammen et al. reported a trend towards a lower AUC for adolescent boys with environment-related adversity (b=-9.37; SE=5.07; p=0.08) compared to their female counterparts (11). In conclusion, ACEs show correlation to diurnal salivary cortisol in most research. While several studies report altered diurnal slopes or cortisol awakening responses, there is little reproducibility of results with authors finding both hypo- and hypercortisolism. This variability suggests that diurnal cortisol patterns may reflect complex, context-dependent adaptations rather than a uniform biomarker signal.

Stress test

Twelve studies measured reactivity of salivary cortisol to a stressor (16–18,21,22,24–26,28,32,33,38), reporting data for 2193 participants (36.8% of cortisol measurements). Different stressors were used, from social or physical standardized stress tests to other specific tasks with saliva samples being collected before and after exposure. Exact timing differed among study groups. Details of sampling are summarized in Table 5. Three studies conducted in the context of psychiatric comorbidity are discussed in section 3.4.3 (17,24,33). A longitudinal cohort study by Bosch et al. revealed that cortisol reactivity varied by the timing of childhood adversity. Pre- and postnatal stress were linked to heightened responses (F=5.17, p=0.006), while late-childhood adversity correlated with higher baseline levels (F=8.08, p=0.005) and adolescent adversity to lower baseline levels (F=4.8, p=0.03). No difference was reported in relation to biological sex (32). Marques-Feixa et al. describe a blunted cortisol response among participants aged 7-17 years exposed to ACEs (F 4.530, p=0.002), despite higher perceived stress (F=9.129, p=0.007) as assessed by the Trier Social Stress Test (TSST) (18). Comparable results are described by Sumner et al. When comparing participants with or without a history of maltreatment in an ANOVA test, F=5.05, p=0.02, with lower levels of cortisol at the second (p=0.07) and third timepoint of measurement (p=0.01).

TABLE 3: Risk of bias assessed using the Newcastle-Ottawa Scale (1: selection, 2: comparability, 3: exposure)

Cohort studies					Case-control studies				
Author	Year	1	2	3	Author	Year	1	2	3
Reichl(25)	2024	**	**	**	Chung (31)	2023	**	**	***
Bhopal (1)	2019	****	**	**	Harkness (29)	2010	****	*	***
Bosch(28)	2012	***	**	****	Marques-Feixa (14)	2021	**	**	**
Bryson (2)	2019	***	**	****	Weems (13,19)	2009	***	*	**
Bryson (16)	2020	***	**	****	Reichl (13)	2019	*	**	*****
Gustafsson (30)	2010	*	*	****	Valentino (36)	2015	***		****
Iob (21)	2021	***	**	***	Weems (20)	2009	***	*	**
Kaess (33)	2017	****	*	****	Peckins (24)	2015	***	*	**
Kuhlman (18)	2015	**	**	***	Isaksson (23)	2013	****	*	*
Kuhlman (22)	2018	***	**	***					
Llorens (27)	2022	***	*	***					
Oriana Linares (34)	2012	**	*	***					
Reichl (26)	2016	**	**	***					
Simmons (4)	2016	****	*	***					
Simmons (15)	2019	*	*	***					
Van Dammen (5)	2020	****	**	****					
Winiarski (17)	2018	***	**	***					
Hartmann do Prado (35)	2017	****	**	***					
Sumner (12)	2014	****		*****					

They found no difference in baseline cortisol, confidence intervals were not reported (16). A twin cohort study of 11-year-olds reported significantly lower cortisol (β=-0.576, p=0.049) in children with ≥3 ACEs, albeit with a small effect size. The strongest association was linked to bullying. This was borderline associated with depressive symptoms at age 21 (25). Peckins et al recruited children between 9 and 12 years old via Child and Family Services and performed a TSST. A blunted cortisol response was more often observed in participants experiencing maltreatment versus healthy controls with statistically significant findings (p<0.05, OR=0.41) (28). According to Winiarski et al., cortisol reactivity moderated the relationship between early life stress and aggression in toddlers (aged 2.5-5.5 years old). A higher rise in cortisol in response to a stress test was associated with more symptoms of aggression only in the presence of early life stress with a coefficient of -0.078 (SE=0.038, p=0.039) (21). In an investigation of adolescent girls from a high-risk population for both childhood adversities and dating perpetration, girls experiencing ACEs showed a significantly higher cortisol reactivity to stress with a steeper cortisol slope (β= 0.947, SE=0.274, p=0.001) (38). Kuhlman et al. demonstrated trauma-specific cortisol responses. Physical abuse was linked to a steeper cortisol peak (p=0.02), while emotional abuse was associated with a slower post-stressor decline (p=0.03) (22). A follow-up study found that the type of abuse influenced psychological symptoms. A raised HPA reactivity combined with physical abuse was associated with more externalizing symptoms (p=0.027) (26). As with the previously discussed diurnal cortisol patterns, cortisol reactivity to stress shows a correlation to ACEs in most studies. However, the direction of this association is inconsistent, with both heightened and blunted responses reported. These findings likely reflect differences in timing, type, and chronicity of adversity, as well as developmental and individual factors.

Psychopathology as co-morbidity

As previously mentioned and documented in literature, experiencing ACEs heightens the risk of developing psychiatric disorders. Evidence from a large population-based study in Canada substantiates this association, demonstrating, a strong correlation between child abuse and various mental conditions (6). In line with these findings, several authors have explored the role of psychopathology on the HPA axis in the presence of ACEs. In a subgroup of children with ADHD, childhood adversity was positively correlated with a higher morning increase in salivary cortisol (r=0.19, p=0.041), which could not be demonstrated in control participants without ADHD diagnosis (27). In a sample of adolescents with ADHD (aged 14-17 years), higher salivary cortisol at awakening was associated with more inattentive symptoms, only in the presence of maltreatment (β=-0.398, p=0.035). In this study group a strong correlation between salivary cortisol and fasting plasma cortisol (p<0.001) was demonstrated (31). Maltreated adolescents with moderate to severe depressive symptoms showed a higher baseline salivary cortisol (p<0.01, η^2=0.08) in research by Harkness et al., but lower cortisol reactivity to stress (p<0.005, η^2=0.64) (33). Weems et al. noted distinct diurnal cortisol patterns in children with PTSD symptoms, including a steeper initial decline and a subsequent rise at night. HLM estimation of a curvilinear pattern resulted in a coefficient of 0.03 when comparing children with vs without PTSD (SE=0.01, p=0.013) (23,24). Children with functional neurological disorder (FND) were investigated by Chung et al. A reduced cortisol awakening response was reported in children with FND, particularly in those with more ACEs using a Pearson correlation test (r=-0.6, p=0.02) (35). Adolescents engaging in NSSI (non-suicidal self-injury) reported significantly higher ACEs related to antipathy and physical maltreatment (p<0.01 and p=0.04 respectively) in comparison to healthy controls. No significant association between hair cortisol and NSSI was observed, nor between salivary cortisol and child abuse. A significant relation of the diurnal slope of salivary cortisol levels to child abuse was found, where adolescents engaging in NSSI had a steeper diurnal slope in the presence of abuse (β=-0.47, p=0.02). On the contrary, healthy controls had a flattened slope although these results did not reach significance (β=0.27, p=0.06) (30). In comparison to their siblings, adolescents engaging in NSSI had higher ACE scores (z=- 2.73, 95% CI -2.05 to -0.34, p<0.01) and HCC levels (z=- 2.57, 95% CI -0.41 to -0.06, p=0.01) (17). Finally, emotional abuse and non-intentional trauma have been established to correlate with internalizing symptoms (respectively p=0.001 and p=0.017) (26). Collectively, we conclude that different psychiatric disorders have an influence on the HPA axis and that this modification shows a correlation to the presence of ACEs. However, the correlation differs among different studies and few patients are included, warranting caution in generalizing conclusions.

Discussion

This review synthesizes the current literature investigating non-invasive cortisol measurement as potential biomarkers for stress in children with ACEs. Across 1,787 hair samples and 4,170 saliva samples, these studies provide valuable insights into the complex relationship between childhood adversity and cortisol dynamics across developmental stages and socio-cultural contexts. Age critically influences cortisol regulation due to maturation of the HPA- axis (1,11,13). Including participants from infancy to adolescence increases generalizability but complicates comparison due to age-related variability. Timing of adversity is an important parameter to consider, as demonstrated by Bosch et al. (32), yet rarely addressed in the existing literature. Gender differences are evident, with for example Van Dammen et al. reporting higher mean HCC in female participants, and Simmons et al. in males (11,13). These sex-specific differences highlight an important limitation in the use of cortisol as a biomarker. Variability in HPA-axis functioning between males and females suggests that cortisol cannot be interpreted uniformly across populations. This limits its utility as a standalone biomarker and underscores the need for age- and sex-specific reference frameworks when considering clinical application. The studies reviewed employed diverse methods to assess ACEs, including standardized questionnaires or study-specific instruments. While these yield cumulative adversity scores, they often fail to capture individual stress perception, limiting precision (11). Distinguishing ACEs from trauma shows a need for standardized tools reflecting their multifaceted nature. Person- and environment-related adversities differently impacted cortisol regulation, underscoring the complexity of assessing childhood adversity. HCC demonstrated strong associations with cumulative adversity, indicating its potential as a biomarker for chronic stress. Previous literature on ACEs has already supported the biology of stress, where physiological stress responses become detrimental when chronically activated (7). Elevated HCC was especially found in younger children, with variations by gender and adversity type. Associations in adolescents were less consistent (1–3,13,19,34,39). Salivary cortisol findings were mixed, with both blunted diurnal slopes or altered AUC metrics, and found elevated cortisol awakening responses (1,11,22,34,37,40). Many lacked statistical significance. Similarly, reactivity to stress tests showed mixed results, with both heightened and blunted reactivity reported, potentially influenced by age, gender, trauma type, and timing (16,18, 21,22,25,32,38) . This suggests limited reliability of salivary cortisol as an ACE biomarker. ACEs-related psychiatric disorders, including NSSI, ADHD, and depression, show distinct cortisol patterns, underscoring the need to account for these comorbidities.

TABLE 4: Demographics of study participants.

Author	Year of publication	Number of patients	Age	Gender	Method of cortisol analysis	Number of samples	Gender
Bhopal	2019	1693	median 12,4m	n/a	Hair	712	371F, 341M
Bryson	2018	603	2y	n/a	Hair	319	186F, 133M
Bryson	2019	500	2-3y	n/a	Hair	297	180F, 117M
Simmons	2018	60	mean 4,25y	34F, 26M	Hair	60	34F, 26M
Simmons	2016	70	mean 9,5y	56F, 14M	Hair	70	56F, 14M
Reichl	2019	64	12-17y	n/a	Hair	63	n/a
Van Dammen	2020	190	12-18y	116F, 74M	Hair	183	n/a
Hartmann do Prado	2017	243	13-17	n/a	Hair	57	15F-15M (case), 18F-9M (control)
Reichl	2016	26	14-18y	24F, 2M	Hair	26	24F, 2M
Bhopal	2019	1350	median 12,4m	n/a	Saliva - diurnal	752	353F, 399M
Valentino	2015	92	3-6y	25F-24M (case) - 23F-20M (control)	Saliva - diurnal	92	25F, 24M (case) - 23F, 20M (control)
Isaksson	2013	418 (197 ADHD /221 HC)	6-17y, mean 11,9y	49F, 148M (case); 129F, 92M (control)	Saliva - diurnal	400	191 (case), 209 (control)
Marques-Feixa	2023	187	7-17y	108F, 79M	Saliva - diurnal	178	n/a
Kuhlman	2015	121	9-16y	59F, 62M	Saliva - diurnal	121	59F, 62M
Weems	2008	41 (26 case/ 15 control)	10-16y	10F, 16M (case), 5F-10M (control)	Saliva - diurnal	41	10F, 16M (case),5F, 10M (control)
Van Dammen	2020	190	12-18y	116F, 74M	Saliva - diurnal	92	n/a
Gustafsson	2010	15	13-19y	14F, 1M	Saliva - diurnal	15	14F, 1M
Llorens	2022	76	14-17	22F, 54M	Saliva - diurnal	76	22F, 54M
Reichl	2016	52	14-18y	48F, 4M	Saliva - diurnal	52	48F, 4M
Reichl	2016	26	14-18y	24F, 2M	Saliva - diurnal	26	24F, 2M
Chung	2023	78 (41 FND/ 37 HC)	11,3-16,1y	n/a	Saliva - awakening response	63	26F, 6M (FND) - 23F 8M (HC)
Kaess	2017	238	14-16	122F, 116M	Saliva -awakening response	69	30F, 39M
Winiarski	2018	219	2,5-5,5y	110F, 109M	Saliva - stress test	219	110F, 109M
Peckins	2015	454	9-12y	212F, 242M	Saliva - stress test	454	212F, 242M
Kuhlman	2015	121	9-16y	59F, 62M	Saliva - stress test	121	59F, 62M
Kuhlman	2018	121	9-16y	57F, 64M	Saliva - stress test	121	57F, 64M
Iob	2021	300	11y	180F, 120M	Saliva - stress test	290	173F, 117M
Bosch	2012	1816	11-21y	926F - 890M	Saliva - stress test	471	245F, 226M
Reichl	2019	64	12-17y	n/a	Saliva - stress test	64	n/a
Harkness	2010	71	12-21y	48F, 23M	Saliva - stress test	71	48F, 23M
Sumner	2014	168	13-17y	94F, 74M	Saliva - stress test	158	n/a
Reichl	2016	52	14-18y	48F, 4M	Saliva - stress test	184	n/a
Oriana Linares	2013	40	mean 16,63y	40F	Saliva - stress test	40	40F
Llorens	2022	76	14-17y	22F, 54M	Fasting blood sample	76	22F, 54M

TABLE 5: Sampling protocols for salivary cortisol.

Author	Year of publication	Type of sampling	Timing of sampling	Type of stressor
Marques Feixa	2023	Saliva - diurnal	1 day; awakening, +30 min, before lunch, before bedtime	
Marques Feixa	2023	Saliva - stress test	stress test at 16:00; -30 min, immediately before stressor, +15 min, + 30 min	Trier Social Stress Test
Chung	2022	Saliva - awakening response	awakening, 30 min after awakening	
Llorens	2022	Saliva - diurnal	2 consecutive days; awakening, +30 min, +60 min, 22:00	
Iob	2021	Saliva - stress test	Baseline, immediately after stressor, +10 minutes	computer game
Van Dammen	2020	Saliva - diurnal	1 weekday; awakening, + 30 min, 12:00, 20:00	
Bhopal	2019	Saliva - diurnal	2 consecutive days; 8:00, 12:00, 16:00	-
Bosch	2019	Saliva - stress test	Pre-test, immediately after, +20 min, +40min	Groninger Social Stress test
Reichl	2019	Saliva - stress test	Before and after CECA interview	CECA interview
Kuhlman	2018	Saliva - diurnal	2 consecutive weekdays; awakening, +45 min, before dinner, before bedtime	
Kuhlman	2018	Saliva - stress test	Baseline at -30min, immediately before stressor, +25min, +35min, +45min, +55min, +65min	Socially-evaluated Cold Pressor Task
Winniarski	2018	Saliva - stress test	Baseline, 20 minutes after completing stress test	Laboratory temperament assessment battery
Kaess	2017	Saliva - diurnal	2 consecutive days; awakening, +30 min, +60 min, 20:00	
Reichl	2016	Saliva - diurnal	3 consecutive days; awakening, +30 min, +60 min, before bed	
Peckins	2015	Saliva - stress test	"-45 min, -10 min, immediately after stressor, +10 min, +20min, +30 min	Trier Social Stress Test
Valentino	2015	Saliva - diurnal	2 consecutive weekend days; awakening, before lunch, before bedtime	
Sumner	2014	Saliva - stress test	test between 14:00 en 19:00; pre-test, immediately after, +20 min	Trier Social Stress Test
Isaksson	2013	Saliva - diurnal	Weekday; awakening, +30min, 16:00, bedtime	
Oriana Linares	2013	Saliva - stress test	"- 45 min, -25 min, -5 min, +20 min, +40 min, +60 min"	Viewing of video of a teen dating argument that escalates into severe physical aggression
Kuhlman	2012	Saliva - stress test	Pre-test, +25min, +35min, +45min, +55min, +65min	Socially-evaluated Cold Pressor Task
Gustafsson	2010	Saliva diurnal	3 weekdays, 8:30, 10:30, 21:00	
Harkness	2010	Saliva - stress test	Baseline, 10 min after first stressor, 20 min after first stressor	Trier Social Stress Test
Weems	2008	Saliva - diurnal	3 days; before breakfast, before lunch, before dinner, before bedtime	

Future studies should examine whether cortisol profiles predict psychiatric risk or can guide interventions targeting HPA-axis dysregulation. The studies reviewed employed diverse methods to assess ACEs, ranging from study-specific questionnaires to validated instruments such as the CTQ, CECA, and ETI. While these tools capture important aspects of adversity, substantial heterogeneity exists in their conceptual scope and operationalization. Importantly, many instruments focus primarily on trauma-related exposures (e.g. abuse and neglect), whereas broader ACE frameworks also include household dysfunction and environmental adversity. Trauma-focused questionnaires may fail to capture the breadth of ACEs, highlighting the need for validated and standardized assessment tools. In addition, ACEs are often summarized as cumulative scores, which improves feasibility but may obscure important dimensions such as timing, chronicity, severity, and developmental context of adversity. This is particularly relevant in paediatric research, where exposure assessment may rely on self-report, proxy report, or study-specific classifications, each with distinct risks of underreporting or misclassification. Importantly, validated tools for childhood victimization do exist, as illustrated by Afifi et al., who used the Childhood Experiences of Violence Questionnaire, a valid and reliable instrument for assessing youth victimization (6). The absence of the original ACE questionnaire (as published by Felliti et al.) in the included studies may reflect its limited applicability in paediatric populations, where proxy reporting and ethical considerations

complicate its use. However, this variability in measurement tools reduces comparability across studies and may contribute to inconsistent findings regarding cortisol associations. This review has several important limitations. First, most included studies were cross-sectional in design, which limits causal inference and hampers the ability to disentangle temporal relationships between adverse childhood experiences and cortisol dynamics. Caution is needed to not overinterpret correlation as causation, as has been highlighted in previous observational research (6,7). Second, substantial heterogeneity was observed in both exposure and outcome assessment. Variability in ACE definitions, measurement instruments, and reporting approaches complicates direct comparison across studies. In parallel, differences in cortisol assessment, including timing of sampling, biological matrices, and laboratory methodologies, may have contributed to inconsistent findings. The wide age range of participants further complicates interpretation, given the developmental changes in HPA-axis functioning throughout childhood and adolescence. Third, selection bias may have influenced the results. Several studies recruited participants through clinical services or social care systems, potentially leading to an overrepresentation of children with more severe adversity or maladaptive coping mechanisms. This may limit the generalizability of findings to broader community populations exposed to less severe or more heterogeneous forms of adversity. Fourth, important confounding factors, including medication use, socioeconomic status, nutritional status, sleep patterns, and diurnal variation, were inconsistently reported and controlled for across studies, further limiting comparability and interpretation. Finally, although the search strategy was predefined, it may not have captured all relevant literature. The absence of broader constructs such as "early life stress" and the limited use of controlled vocabulary (e.g., MeSH terms) may have reduced the sensitivity of the search. Future research should explore mediators and moderators, including genetics, parenting styles, social support, and psychiatric symptoms, and prioritize longitudinal designs to clarify the long-term impact of ACEs on cortisol dynamics. Standardized cortisol sampling and analysis protocols are needed to improve comparability and clinical utility, and interdisciplinary collaboration is essential to translate these findings into practice. Cortisol may help identify ACE-related risk but cannot capture the full complexity of adversity alone. HCC shows promise as a retrospective marker of cumulative stress. However, its clinical use requires further validation, and distinguishing adaptive from maladaptive responses remains challenging, as both hypo- and hypercortisolism occur following adversity. Particularly in saliva, non-invasively measured cortisol is unlikely to serve as a reliable standalone biomarker for ACEs. Instead, it may be more informative as part of a multi-biomarker framework integrating physiological, psychological, and environmental measures. Subsequently, trends on a community or population-based level may be more of interest than the individual's value. This is in line with critiques of the ACE framework as a probabilistic, population-level construct rather than a tool to diagnose individual vulnerability. When using the ACEs framework for policy making, caution should be taken to avoid stigmatization of families and vulnerable children (7).

Conclusion

This systematic review highlights the complex impact of childhood adversity on cortisol regulation and underscores the utility of non-invasive cortisol measurements, particularly HCC, as biomarkers for chronic stress. By advancing our understanding of the physiological pathways linking early adversity to health outcomes, these findings might provide a foundation for targeted interventions to mitigate the long-term effects of ACEs on physical and mental well-being. The clinical application of cortisol as a diagnostic or prognostic tool requires further refinement, including standardization of measurement protocols and a deeper understanding of individual variability in stress responses.

Statement

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