

Dental caries linked to gestational and early-life secondhand smoke exposure

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ABSTRACT

Background: Few studies have investigated how different exposure windows - gestational, early postnatal, and current - of secondhand smoke (SHS) contribute to early childhood caries (ECC). Understanding these critical windows is essential for designing targeted interventions. We evaluated the association between SHS exposure and ECC among preschoolers.

Method: This study was nested within the prospective birth cohort (HAPIN trial) conducted in southern India (2018–2020). Comprehensive data on exposure, lifestyle, and health were collected at multiple maternal and child time points. Between April and September 2023, dental caries and current exposure were assessed in children aged 3–5 years by a trained dentist using standardized, calibrated methods. SHS exposure was measured using questionnaires and urinary nicotine metabolites (cotinine, 3-hydroxycotinine). Negative binomial hurdle models were used to assess associations between SHS exposure and ECC prevalence.

Results: Among 712 participants (mean age: 4.01 ± 0.45 years; 53.4% boys), 478 children (67.2%) were diagnosed with ECC. Children exposed to SHS (urinary nicotine equivalents - sum of cotinine and 3-hydroxycotinine) during the gestational period demonstrated significantly higher prevalence of caries than their unexposed counterparts, with an incidence rate ratio (IRR) of 1.29 (95% CI, 1.18–1.42); second trimester was identified as a critical exposure window. Similarly, postnatal exposure increased ECC risk (IRR, 1.19; 95% CI, 1.08–1.31). Paternal smoking significantly elevated ECC prevalence, with children whose fathers smoked ≥11 cigarettes/day showing higher odds of ECC (odds ratio (OR), 1.90; 95% CI, 1.10–3.41). Urinary nicotine metabolites exhibited a dose-response relationship with ECC, showing the strongest association during pregnancy. Children classified as slow metabolizers were more vulnerable to third-trimester SHS exposure (OR, 1.74; 95% CI, 1.15–2.64).

Conclusion: SHS exposure from pregnancy through early childhood was linked to higher odds of developing dental caries.

Clinical significance: Findings underscore another precarious health risk associated with SHS exposure and highlight the need for integrated oral–environmental health prevention strategies.

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1. Introduction

Early Childhood Caries (ECC) is the most prevalent chronic childhood disease. Despite decades of research on its multifactorial etiology and preventive strategies, its burden persists unabated, affecting an estimated 600 million children worldwide [1]. ECC disproportionately affects racially and ethnically minoritized groups and children living in low- and middle-income countries (LMICs), reflecting entrenched inequities in access to dental care, financial barriers, workforce shortages, and limited oral health literacy. In India, prevalence ranges from 42% to 63% [1], underscoring its status as a major public health concern. Consequently, ECC continues to impose profound ramifications on oral and general health, quality of life, and incurs considerable social and economic costs on families and healthcare systems [2]. These disparities highlight the need to investigate upstream and potentially modifiable risk factors beyond individual behaviors.

Secondhand smoke (SHS) is a pervasive environmental pollutant, with no safe level of exposure. Although public smoking bans have led to measurable declines in exposure, domestic environments remain the primary source, with over 40% of children globally residing in households with at least one smoker [3]. While the systemic health impacts of SHS are well documented [3–6], its influence on dental development is comparatively underexplored. Socioeconomic and behavioral factors such as parental education, household income, oral hygiene practices, and dietary habits are known ECC determinants [2,7,8], and are often concomitant with tobacco smoke exposure, compounding children's vulnerability. A significant body of evidence links SHS exposure to higher ECC risk, though effect estimates vary across studies due to differences in participants' characteristics (age, race/ethnicity, hormones, and body composition), exposure definition, intensity and timing, exposure assessment methods (self-report vs. biomarkers), biomarker cut-points to classify exposure, and ECC risk assessment [9–12]. Importantly, biomarker-defined SHS exposure captures internal dose and timing, which are not equivalent to household smoking reports and may relate differently to ECC initiation and severity.

The Developmental Origins of Health and Disease framework posits that exposure to environmental insults during sensitive developmental periods can permanently reshape organ maturation and long-term health trajectories [13]. Odontogenesis commences around six weeks of gestation, with matrix deposition and mineralization largely completed by the first year [14,15]. Given that tobacco smoke compounds readily traverse the placenta and infants exhibit rapid breathing rates and frequent hand-to-mouth behaviors [16], SHS exposure during these critical windows may heighten caries susceptibility. However, most existing studies rely on single or self-reported exposure measures, missing fluctuations across the nuanced stages of tooth development and maturation. Therefore, identifying whether exposure during specific periods confers differential risk is imperative for informing targeted interventions.

To delineate these temporal windows of exposure, we leveraged data from the Household Air Pollution Intervention Network (HAPIN) cohort. Using repeated urinary measurements of cotinine and 3-hydroxycotinine (3-HC), we objectively quantified exposure from pregnancy through early childhood and evaluated its independent and cumulative effects on caries burden.

2. Methods

2.1. Study population

Our analytic sample included participants enrolled from 2018 to 2020 in the Villupuram and Nagapattinam districts of Tamil Nadu (India), one of four international research sites of the HAPIN trial (NCT02944682), a maternal-child cohort, described in detail elsewhere [17,18]. Briefly, pregnant women were eligible to participate if they had a confirmed singleton pregnancy between 9 and 20 weeks of gestation,

planned to reside in the study area for 12 months post-recruitment, and primarily used biomass fuel for cooking. Pregnant women who were smokers were excluded. Questionnaires and biological samples pertaining to exposures, lifestyle, and health were collected at various time points during pregnancy and after.

All participants provided written informed consent. The Caries Risk from exposure to Environmental tobacco Smoke (CARES) study protocol was approved by institutional review boards at Emory University (00089799) and Sri Ramachandra Institute of Higher Education and Research (IEC-NI22/JUL/83/82). Reporting complied with STROBE guidelines.

2.2. Exposure measurement

During onsite interviews, trained bilingual staff assessed SHS exposure at multiple time points: pregnancy, early childhood, and current ECC assessment period. Missing or ambiguous data were minimal (<3% for any variable) and verified through follow-up telephone calls. Consequently, the complete-case approach did not result in participant exclusion or alter the sample size. We defined a child as having SHS exposure when any family member living with the child reported smoking cigarettes, cigars, or pipe smoke. We grouped self-reported SHS exposure by period (e.g., in utero to post-birth) into three categories: unexposed, ever exposed, and always exposed (details in Supplementary Material). We also examined current paternal smoking, categorizing the number of cigarettes smoked per day (CPD) into three groups: 0, 1 to 10, and ≥ 11 . At each visit, the smoking status of all household members, including female caregivers such as grandmothers, aunts, and other adult women, was recorded. No active smoking was reported among these individuals during the study period, and thus this subgroup was not analyzed separately.

Urine samples were collected from pregnant women in each trimester and children during postnatal visits (3, 6, 12, and 36 to 60 months). Urinary concentrations of cotinine and 3-HC were quantified by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) [19,20] (details in Supplementary Material). Limit of detection (LOD) was 0.05 ng/mL for cotinine and 3-HC (Appendix Tables 1 and 2). Urine concentrations were normalized using creatinine. Nicotine equivalents (NE2) were calculated as cotinine + 3-HC, and the nicotine metabolite ratio (NMR) was determined as 3-HC/cotinine [21].

2.3. Outcome assessment

Clinical data on caries were collected once from children aged 3 to 5 years, following standardized methods by a single trained and calibrated pediatric dentist (see Supplementary Methods for reliability metrics) to ensure consistency. Data were summed from all primary tooth surfaces to calculate the dmfs index for each child, wherein 'd' component was defined by International Caries Detection and Assessment System (ICDAS), filled surfaces (f), and missing surfaces owing to caries (m). Children were classified by oral health status as Caries-Free (CF, ICDAS = 0), Noncavitated Lesions (NCL, ICDAS = 1, 2 on ≥ 1 tooth), or Cavitated Lesions (CL, ICDAS = 3–6 on ≥ 1 tooth, with or without non-cavitated lesions). Analyses treated dmfs as a count outcome; CF, NCL, and CL classifications were used solely for descriptive purposes. We also assessed several factors associated with ECC development, such as dietary behavior (feeding history, sugar consumption, between-meal snack frequency), oral hygiene behavior (toothbrushing frequency, parental supervision of brushing, fluoride toothpaste usage), and last dental visit.

2.4. Covariates

Potential covariates were selected a priori for known or plausible associations with both SHS exposure and ECC, as well as independent

Table 1
Distribution according to caries incidence (Mean $\pm$ SD or n (%)) among CARES-HAPIN children (2023-2025, Tamil Nadu, India).

Characteristic	Overall (N=712)	Children with no caries lesions (CF, N=234)	Children with non- cavitated lesions (NCL, N=97)	Children with cavitated lesions (CL, N=381)
Children's demographic information				
Age, years	4.01 (0.45)	3.9 (0.44)	3.9 (0.42)	4.0 (0.45)
Gender				
Boy	380 (53.4)	119 (50.8)	51 (52.6)	210 (55.1)
Girl	332 (46.6)	115 (49.2)	46 (47.4)	171 (44.9)
BMI	13.03 (1.60)	13.10 (1.62)	13.1 (1.60)	12.9 (1.59)
Parent's demographic information				
Mother's age, years	23.98 (3.74)	23.85 (3.87)	24.3 (4.0)	23.9 (3.6)
Mother's education				
No formal education	255 (35.8)	103 (44.0)	26 (26.8)	126 (33.1)
Primary school completed	198 (27.8)	58 (24.8)	33 (34.0)	107 (28.1)
Secondary school or college completed	259 (36.4)	73 (31.2)	38 (39.2)	148 (38.8)
Father's education				
No formal education	273 (38.4)	94 (40.2)	34 (35.1)	145 (38.1)
Primary school completed	222 (31.2)	77 (32.9)	34 (35.1)	111 (29.1)
Secondary school or college completed	212 (29.8)	61 (26.1)	28 (28.9)	124 (32.5)
Household food insecurity				
Secure	583 (81.9)	187 (79.9)	81 (83.5)	315 (82.7)
Mild insecurity	99 (13.9)	40 (17.1)	13 (13.4)	46 (12.1)
Moderate or severe insecurity	30 (4.2)	07 (3.0)	03 (3.1)	20 (5.2)
Dietary diversity				
Low	558 (78.4)	196 (83.8)	76 (78.3)	286 (75.1)
Medium	131 (18.4)	34 (14.5)	15 (15.5)	82 (21.5)
High	23 (3.2)	04 (1.7)	06 (6.2)	13 (3.4)
Mode of birth delivery				
Vaginal	493 (69.2)	165 (70.5)	67 (69.1)	261 (68.5)
Caesarean	217 (30.5)	69 (29.5)	29 (29.9)	119 (31.2)
Other	02 (0.3)	0 (0)	01 (1.0)	01 (0.3)
Preterm birth				
Yes	45 (6.3)	16 (6.8)	04 (4.1)	25 (6.6)
No	667 (93.7)	219 (93.2)	93 (95.9)	356 (93.4)
Low birth weight				
Yes	283 (39.7)	92 (39.3)	38 (39.2)	153 (40.2)
No	429 (60.3)	142 (60.4)	59 (60.8)	228 (59.8)
Self-reported prenatal SHS exposure				
Yes	254 (35.6)	77 (32.9)	27 (27.8)	150 (39.4)
No	458 (64.4)	157 (67.1)	70 (72.2)	231 (60.6)
Self-reported postnatal SHS exposure				

Table 1 (continued)

Characteristic	Overall (N=712)	Children with no caries lesions (CF, N=234)	Children with non- cavitated lesions (NCL, N=97)	Children with cavitated lesions (CL, N=381)
Yes	277 (38.9)	80 (34.2)	30 (30.9)	167 (43.8)
No	435 (61.1)	154 (65.8)	67 (69.1)	214 (56.2)
Self-reported current SHS exposure				
Yes	200 (28.1)	60 (25.6)	19 (19.6)	121 (31.7)
No	512 (71.9)	174 (74.4)	78 (80.4)	260 (68.3)
Children's dietary and oral hygiene habits				
First sugar intake				
<12 months	422 (59.3)	78 (33.3)	68 (70.1)	276 (72.4)
$\geq$ 12 months	290 (40.7)	156 (66.7)	29 (29.9)	105 (27.6)
Frequency of snacking				
Once a day	486 (68.3)	180 (76.9)	55 (56.7)	251 (65.9)
Twice a day	226 (31.7)	216 (23.1)	42 (43.3)	130 (32.1)
Fed after brushing				
Yes	391 (54.9)	115 (49.1)	54 (55.6)	222 (58.3)
No	321 (45.1)	119 (50.9)	43 (44.3)	159 (41.7)
Frequency of child brushing				
Once a day	314 (44.1)	54 (23.0)	43 (44.3)	217 (56.9)
Twice a day	398 (55.9)	180 (76.9)	54 (55.7)	164 (43.0)
Type of toothpaste				
No toothpaste	21 (2.9)	03 (1.3)	03 (3.1)	15 (3.9)
Nonfluoridated toothpaste	263 (36.9)	121 (51.7)	54 (55.7)	88 (23.1)
Fluoridated toothpaste	364 (51.1)	99 (42.3)	32 (32.9)	232 (60.9)
Uncertain	64 (9.1)	11 (4.7)	08 (8.3)	45 (12.1)

SD - standard deviation

determinants of ECC. A directed acyclic graph (DAG) was constructed to delineate relationships among variables and distinguish confounders from causal intermediates (Appendix Fig. 1). Final models were adjusted for socioeconomic status (SES) index (continuous), child's age at dental evaluation (continuous), gender (male/female), child's body mass index (BMI, continuous), timing of first sugar intake (categorical), snacking frequency (once/twice daily), and fluoridated toothpaste use (categorical). The SES index was derived using principal component analysis as a composite measure of household water and sanitation quality, electricity access, household size, food insecurity, maternal education, and housing materials (floor, wall, and roofing). To balance model parsimony, backward stepwise elimination minimizing Akaike's Information Criterion (AIC) was applied, retaining predictors associated with dmfs at $p < 0.10$ in either the count or zero component of the hurdle model. Theoretically relevant variables (child's BMI, gender, age, and SES) were retained irrespective of statistical significance. To assess temporal influence on the association with dental caries, time-specific models were specified for each exposure window, with prenatal exposure included as a covariate in postnatal models.

2.5. Statistical analyses

All analyses were performed in R (version 4.4.1). Participant

Table 2
Negative binomial hurdle model estimates for ECC risk across quartiles of tobacco biomarker exposure among CARES-HAPIN Children (2023–2025, Tamil Nadu, India).

Timepoint	N	Count part		Zero part	
		Coefficient (SE)	p-value	Coefficient (SE)	p-value
Average Prenatal Cotinine ^a					
Log Linear	712	0.19 (0.04)	<0.001	0.12 (0.09)	0.238
Quartile 2 ^c	178	-0.02 (0.11)	0.879	0.51 (0.26)	0.047
Quartile 3 ^c	178	0.21 (0.11)	0.048	0.36 (0.25)	0.152
Quartile 4 ^c	178	0.43 (0.11)	<0.001	0.21 (0.25)	0.395
Average Postnatal Cotinine ^{a,b}					
Log Linear	705	0.15 (0.04)	<0.001	0.29 (0.09)	0.002
Quartile 2 ^c	176	0.06 (0.11)	0.560	0.77 (0.26)	0.003
Quartile 3 ^c	176	0.11 (0.11)	0.318	0.57 (0.26)	0.027
Quartile 4 ^c	176	0.35 (0.11)	0.001	0.72 (0.27)	0.007
Average Prenatal 3-hydroxycotinine ^a					
Log Linear	712	0.22 (0.04)	<0.001	0.20 (0.09)	0.081
Quartile 2 ^c	178	0.12 (0.11)	0.256	0.41 (0.25)	0.101
Quartile 3 ^c	178	0.25 (0.11)	0.019	0.17 (0.25)	0.494
Quartile 4 ^c	178	0.48 (0.10)	<0.001	0.48 (0.26)	0.062
Average Postnatal 3-hydroxycotinine ^{a,b}					
Log Linear	705	0.13 (0.04)	0.006	0.36 (0.09)	0.001
Quartile 2 ^c	176	-0.03 (0.11)	0.795	0.56 (0.26)	0.030
Quartile 3 ^c	176	0.03 (0.11)	0.751	0.74 (0.26)	0.004
Quartile 4 ^c	176	0.21 (0.11)	0.050	0.61 (0.26)	0.020
Average Prenatal Nicotine Equivalents ^a					
Log Linear	712	0.26 (0.04)	<0.001	0.18 (0.09)	0.150
Quartile 2 ^c	178	-0.06 (0.11)	0.567	0.41 (0.25)	0.109
Quartile 3 ^c	178	0.21 (0.11)	0.048	0.17 (0.25)	0.496
Quartile 4 ^c	178	0.41 (0.10)	<0.001	0.26 (0.26)	0.308
Average Postnatal Nicotine Equivalents ^{a,b}					
Log Linear	705	0.17 (0.04)	0.001	0.39 (0.09)	0.001
Quartile 2 ^c	176	0.02 (0.11)	0.843	0.57 (0.25)	0.024
Quartile 3 ^c	176	0.06 (0.10)	0.592	0.99 (0.27)	<0.001
Quartile 4 ^c	176	0.31 (0.11)	0.004	0.55 (0.26)	0.033

^a Adjusted for socioeconomic status index, child's age at dental evaluation, gender, body mass index, timing of first sugar intake, frequency of snacking and use of fluoridated toothpaste
^b Additionally adjusted for prenatal levels
^c Quartile 1 is the referent

characteristics were summarized using means and standard deviations (SD) for continuous variables and counts (%) for categorical variables. Intra-examiner agreement was assessed via Cohen's kappa. Geometric means were calculated for cotinine, 3-HC, and NE2 to summarize skewed distributions; concentrations below the LOD were imputed as LOD/ $\sqrt{2}$. Right-skewed biomarkers were natural log-transformed prior to analyses. Analyses were restricted to observations with complete urinary biomarker data at each time point. Average pre- and postnatal exposures were defined as the mean of trimester-specific and early-life visit (3, 6, 12 months) concentrations, respectively; current exposure (36–60 months) reflected levels at sample collection. The dmfs distribution (Appendix Fig. 2) exhibited overdispersion with an excess zero (mean = 10.9, SD = 13.1). Consequently, we employed a negative binomial hurdle (NBH) model [22]. Urinary tobacco biomarkers were divided into quartiles with the first quartile as the reference. Additionally, to examine potential nonlinear dose–response relationships between SHS and ECC (presence vs. absence), restricted cubic splines with three knots were applied [23] (Appendix Figs. 3–5). Based on prior studies, NMR validity for predicting nicotine exposure is greatest when comparing the lowest quartile to the top three quartiles [24,25]. This cut-off was used to classify slow vs. normal/fast metabolizers.

3. Results

3.1. Study population

Of 735 eligible children, 712 (96.9%) consented to participate in the

study. Table 1 summarizes demographic characteristics stratified by caries status. The mean age at ECC assessment was 4.01 ± 0.45 years with 53.4% being boys. Dental caries was detected in 32.8% of children with CF, 13.6% with NCL, and 53.6% with CL (details in Supplementary Material).

3.2. Urinary nicotine metabolite profiles

Appendix Table 3 depicts GMs and 95% confidence intervals of urinary concentrations of cotinine, 3-HC, and NE2 across select demographic categories. For prenatal SHS exposure, GMs were as follows: cotinine: 1.65 µg/g creatinine, 3-HC: 4.32 µg/g creatinine, and NE2: 6.32 µg/g creatinine. Postnatal SHS exposure showed higher concentrations, with GMs of cotinine: 5.21 µg/g creatinine, 3-HC: 9.49 µg/g creatinine, and NE2: 15.87 µg/g creatinine. Distinct nicotine metabolite profiles were observed, with 3-HC predominating among mothers and cotinine more prevalent in children. No significant differences were noted between boys and girls.

3.3. Association between SHS exposure and ECC

Filled surfaces were infrequent in this cohort (n < 30), reflecting limited treatment access rather than true disease absence, rendering formal analysis infeasible. The NBH model, adjusted for confounders (Appendix Table 4, Fig. 1), revealed a robust association between elevated first-trimester cotinine levels and caries burden, with an IRR of 1.10 per log-unit increment (95% CI, 1.05–1.15). This trend persisted, though diminished, in the second (IRR, 1.05; 95% CI, 0.99–1.12, borderline) and third (IRR, 1.08; 95% CI, 1.01–1.14) trimesters, per log-unit increase in cotinine concentration. Average prenatal exposure yielded the greatest effect (IRR, 1.20; 95% CI, 1.12–1.30). Average postnatal exposure independently contributed to ECC risk (IRR, 1.16; 95% CI, 1.08–1.25) per one-unit increase, with distinct estimates at six months (IRR, 1.08; 95% CI, 1.02–1.15), twelve months (IRR, 1.09; 95% CI, 1.03–1.16), and a marginal effect at 36–60 months (IRR, 1.15; 95% CI, 0.98–1.36). The zero-component corroborated these findings: each log-unit increment in average postnatal cotinine exposure amplified the likelihood of ECC onset (OR, 1.34; 95% CI, 1.12–1.61) while average prenatal exposure showed no significant association (OR, 1.13; 95% CI, 0.92–1.38). A cognate yet more pronounced pattern emerged for 3-HC, where each log-unit increase in average prenatal levels corresponded to an IRR of 1.24 (95% CI, 1.14–1.36), with significant effects across the first (IRR, 1.10; 95% CI, 1.04–1.16), second (IRR, 1.09; 95% CI, 1.02–1.15), and third trimesters (IRR, 1.10; 95% CI, 1.03–1.17). Average postnatal exposure was similarly consequential, yielding an IRR of 1.13 (95% CI, 1.04–1.24) per one-unit increment, with the six-month period exhibiting the strongest zero-hurdle effect (OR, 1.16; 95% CI, 1.01–1.33). For nicotine equivalents, consistent associations were observed with caries prevalence across the first (IRR, 1.13; 95% CI, 1.07–1.20), second (IRR, 1.11; 95% CI, 1.04–1.19), third trimesters (IRR, 1.12; 95% CI, 1.04–1.20) and at six months postnatally (IRR, 1.07; 95% CI, 1.00–1.14, marginal) per log-unit increase in exposure. Zero-component analyses accentuated their broader impact: second trimester, six-month, and average postnatal NE2 exposures exacerbated the probability of ECC by 16% (OR, 1.16; 95% CI, 0.99–1.37, borderline), 20% (OR, 1.20; 95% CI, 1.02–1.41), and 47% (OR, 1.47; 95% CI, 1.16–1.86), respectively. These findings indicate that early-life SHS exposure not only augments ECC burden but also heightens the probability of onset. Results remained consistent under alternative covariate specifications, substantiating the robustness of these associations (Appendix Tables 5 and 6).

Although none of the mothers reported smoking, paternal and grandfather smoking significantly impacted ECC risk. Children with fathers who smoked 11 or more CPD had a markedly higher prevalence of ECC (77.9%) compared to those with no paternal smoking (64.9%), with an adjusted OR of 1.90 (95% CI, 1.10–3.41; p = 0.015). Those

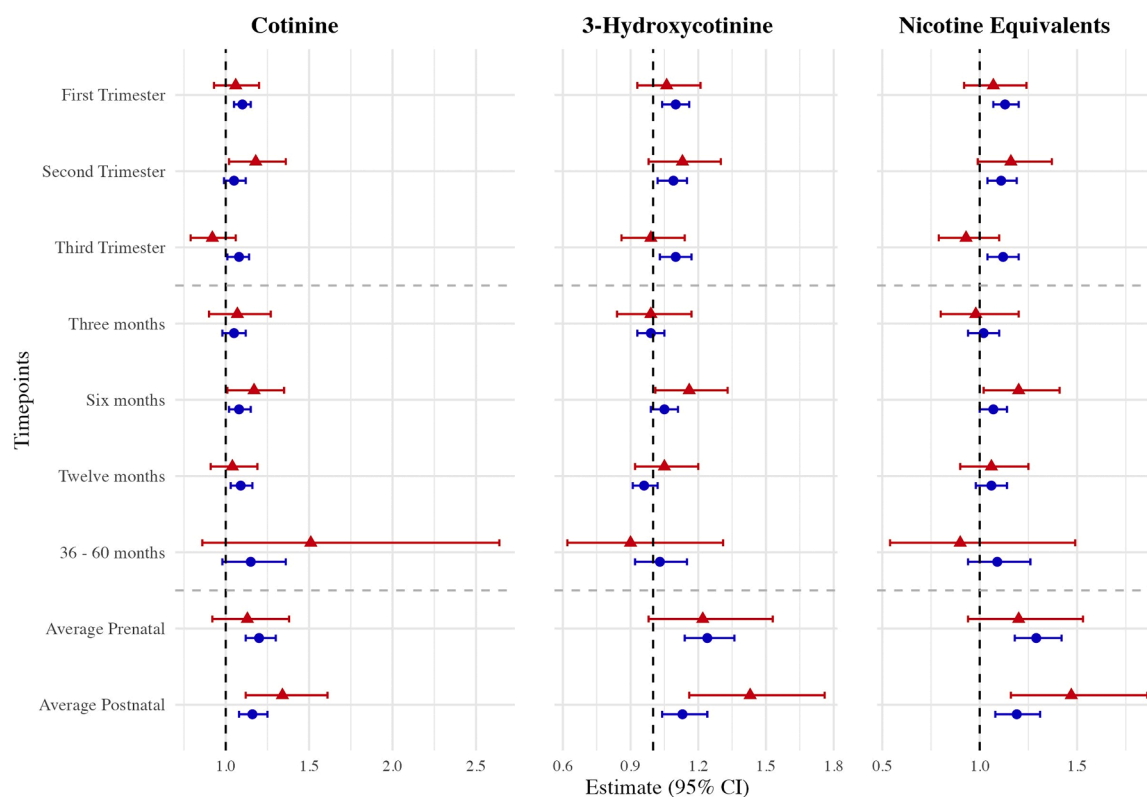


Fig. 1. Forest plot for cotinine, 3-hydroxycotinine and nicotine equivalents concentrations at various time points among CARES-HAPIN children.

*Incident rate ratios (IRR, blue) and odds ratio (OR, red) are shown with error bars representing 95% confidence intervals (CIs), with the dashed line at 1 indicating no association. IRRs >1 indicate higher rates of dental caries and ORs <1 indicate lower odds of being caries-free. Estimates are adjusted for (a) socioeconomic status index, child's age at dental evaluation, gender, body mass index, timing of first sugar intake, frequency of snacking and use of fluoridated toothpaste, and (b) additionally average prenatal levels

whose fathers smoked 1–10 CPD also presented an elevated prevalence (66.7%), though the association was not statistically significant (OR, 1.08; 95% CI, 0.77–1.50; $p = 0.317$). Additionally, the number of smokers in the household further compounded ECC risk, with children exposed to two smokers having an 89.4% prevalence compared to 62.1% for those with one smoker (Appendix Table 7). These findings suggest a dose-dependent relationship of paternal smoking and household smoking intensity on ECC risk. To assess the additive effect of SHS exposure on ECC, children were classified into four categories (Appendix Table 8). Compared to children with no SHS exposure, those exposed to both prenatal and postnatal SHS had higher odds of developing ECC (OR, 1.35; 95% CI, 1.00–2.05; $p = 0.028$).

SHS exposure during the third trimester was significantly associated with an increased ECC risk in slow metabolizers (OR, 1.74; 95% CI, 1.15–2.64; $p = 0.009$) (Appendix Table 9). No significant associations were detected for other exposure periods.

3.4. Categorical analyses

While the quartile estimates in Table 2 did not exhibit a strictly monotonic gradient, the continuous exposure metrics, which assess the overall linear trend, were predominantly positive and significant. In the count model, children in quartile 4 of prenatal cotinine demonstrated a 54% greater caries burden than the reference quartile. Postnatal cotinine followed a congruent pattern, resulting in a 16% increase in ECC per interquartile range (β , 0.15; SE, 0.04) and a marked 42% rise at the highest quartile. For 3-HC, prenatal quartiles 3 and 4 correlated with 28% (β , 0.25; SE, 0.11) and 62% (β , 0.48; SE, 0.10) heightened ECC risk, respectively. Postnatal 3-HC quartile-based estimates were comparatively attenuated. In the zero-inflation model, higher cotinine concentrations were inversely related to the probability of remaining caries-

free. Prenatal exposure in quartile 2 conferred a 65% elevated odds of ECC (β , 0.51; SE, 0.26) whereas postnatal exposure exerted stronger effects: quartiles 2 to 4 were associated with approximately twofold higher odds of caries. For 3-HC, prenatal exposure showed a marginal association in quartile 4. In contrast, postnatal quartiles 3 and 4 were associated with 2.1- and 1.8-fold increases in the odds of having caries (β , 0.74; SE, 0.26; β , 0.61; SE, 0.26), respectively. Total NE2 levels substantiated these findings; children in quartile 3 of postnatal exposure exhibited the strongest effect, with a 2.7-fold increase in odds (β , 0.99; SE, 0.27), whereas quartile 4 corresponded to a 73% reduction in the likelihood of being caries-free (β , 0.55; SE, 0.26). No significant associations were found for prenatal NE2.

4. Discussion

This study provides longitudinal, biomarker-based evidence that both prenatal and early-life SHS exposure increase the risk of ECC. The strongest associations were observed during the second trimester, indicating a critical exposure window. These effects persisted into early childhood, with a modest postnatal signal around six months, underscoring the enduring influence of SHS on developing dentition.

Self-reported SHS exposure is prone to underestimation. In our cohort, 35.6% of mothers reported SHS exposure during pregnancy and 38.9% during the child's first year, substantially lower than biomarker-based estimates, suggesting systematic underreporting. In concordance with previous studies using salivary or urinary cotinine [26,27], our findings corroborate the association between SHS exposure and elevated ECC risk, albeit with attenuated magnitude.

The temporal specificity of these associations reinforces their biological plausibility. Across exposure metrics, the prenatal (mid-gestational trimester) period exhibited a convergent pattern with consistent

elevations in the count component of the hurdle model, demonstrating that maternal SHS exposure increased caries burden. In contrast, the associations with the probability of remaining caries-free were equivocal. This period corresponds to enamel maturation and mineralization [10,11].

At the cellular level, multiple well-established biological and toxicological pathways elucidated in the literature offer a coherent rationale for these associations. Tobacco compounds that cross the placenta may perturb cellular microenvironments critical for enamel formation. SHS contains more than 10^{16} free radicals per cigarette, comprising reactive oxygen species (ROS) and peroxides [28,29], which induce substantial oxidative stress in developing dental tissues. Such stress may cause direct epithelial injury and dysregulate pathways governing cell proliferation, differentiation, and proinflammatory cytokines via activation of nuclear factor- κ B (NF- κ B) and activator protein 1 (AP-1). Beyond its canonical pathway, NF- κ B has been implicated in the regulation of odontogenesis [30]. Experimental studies in dental pulp stem cells demonstrate that activation of the NF- κ B p65 subunit upregulates expression of genes integral to mineral deposition and extracellular matrix remodelling, including RUNX2, Osterix, Osteocalcin, BMP4, PTX3, and Matrix Metalloproteinases [31]. As this signaling cascade is normally rapid and tightly regulated, its prolonged or aberrant activation in response to exogenous toxic insults may plausibly interfere with enamel maturation [31–33].

Concurrently, oxidative damage to DNA, lipids, and proteins can propagate secondary ROS; consistent with this mechanism, SHS exposure has been associated with elevated 8-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA injury. These molecular perturbations can impair ameloblast function via oxidative stress, altered calcium homeostasis, or hypoxic insult. This abnormal oxygen tension can alter enamel matrix pH and inhibit proteolytic enzyme activity, interfering with the regulated sequence of matrix turnover and crystal growth, ultimately leading to hypomineralized enamel. Correspondingly, a parallel elevation in risk at six months suggests that early infancy is another window of vulnerability, coinciding with tooth eruption and initial microbial colonization that may facilitate biofilm formation on developing enamel. Postnatal SHS exposure was linked to both components of the hurdle model, implying a dual influence on caries initiation and subsequent progression. Notably, it remained associated with ECC after adjustment for prenatal exposure, dietary sugar, and oral hygiene. This divergence implies that prenatal exposure primarily compromises enamel structural integrity, whereas postnatal exposure additionally modulates the ecological and microbial milieu driving caries initiation.

The non-monotonic pattern evident for postnatal nicotine equivalents in the zero component likely reflects a plateau or diminution at the highest quartile rather than a substantive departure from the underlying exposure–response relationship. Despite adjustment for key covariates, systematic differences in characteristics may have persisted, contributing to this phenomenon. More broadly, such trends have been well documented in epidemiologic research and may arise from interrelated mechanisms, inclusive of heterogeneity, depletion of susceptible individuals, exposure misclassification, and co-varying risk factors across exposure strata [34,35].

Associations between pre- and postnatal SHS exposure and ECC persisted after accounting for socioeconomic context; these findings are unlikely to be explained by differences in household resources or health-related behaviors between smoking and non-smoking families. Though such behaviors may lie on the causal pathway, their inclusion did not meaningfully alter effect estimates, consistent with a direct contribution of SHS to caries development.

Nicotine and its metabolites promote *Streptococcus mutans* adhesion and virulence by upregulating glucosyltransferase and glucan-binding protein genes, suppressing immune defenses, depleting salivary antioxidants such as vitamin C, and reducing salivary IgA and buffering capacity [36–40]. SHS also contains heavy metals, including cadmium, an independent risk factor for early caries [41], which may further

compound microbial and host-mediated effects. This may shift the equilibrium between remineralization and demineralization toward net mineral loss.

Nicotine is primarily metabolized to cotinine and 3-HC via CYP2A6 [42–44]. Nicotine metabolite ratio thus serves as a phenotypic marker of CYP2A6 enzyme activity. Lower NMR values indicate reduced enzyme activity and slower nicotine metabolism [45]. Because environmental and hormonal factors contribute to NMR variability, this phenotype may be more informative than genotype alone [46,47]. Our analysis suggests that slow metabolizers may be more prone to SHS-related effects on ECC, owing to slower depuration of nicotine metabolites and extended fetal and infant exposure duration. Population-level data on CYP2A6 variants are lacking for our region, and we did not perform genotyping; future work should incorporate genetic characterization to elucidate inter-individual variability.

Taken together, our results suggest that SHS may directly impair the developing dentition, with effects spanning gestational and early childhood developmental periods. Identification of these critical exposure periods offers strategic intervention points: integrating SHS avoidance counseling into antenatal care and early childhood oral health programs may represent a pragmatic and cost-effective approach in resource-limited settings.

Key strengths include biomarker-based exposure assessment using both cotinine and 3-HC, rarely evaluated simultaneously in prior SHS–ECC research; a sizable longitudinal sample with repeated measures; and the use of the negative binomial hurdle model, which rigorously accounts for excess zeros and overdispersion in caries counts. Repeated biomarker assessments minimize temporal ambiguity and, when integrated with contemporaneous self-reported exposure data, strengthen causal inference. While experimental confirmation is not feasible, our design aligns with several of Hill's criteria—temporality, dose–response, plausibility, and internal consistency, providing cogent evidence that SHS may contribute to ECC [48]. Nevertheless, these inferences necessitate caution, as SHS typically co-occurs with pollutants such as carbon monoxide, polycyclic aromatic hydrocarbons, and volatile organic compounds, which may act as confounders. The absence of mixture-based analyses precludes the definitive isolation of SHS effects from these correlated environmental confounders, warranting further investigations.

Beyond this, several caveats merit consideration: although a proportion of associations attained statistical significance, a subset of estimates demonstrated borderline confidence intervals, indicative of limited precision and necessitating circumspect interpretation. Furthermore, the absence of data on multi-unit housing—where smoke infiltration from neighboring units remains a risk—alongside potential residual confounding, and the use of sample-specific biomarker quartiles, may constrain generalizability.

5. Conclusion

Urinary nicotine biomarkers may provide critical insights into ECC risk beyond self-reported SHS exposure. Our results accentuate the need for both timing and magnitude of SHS exposure in shaping childhood oral health, with the second trimester emerging as a sensitive window and a distinct postnatal signal around six months. Associations with paternal smoking and increased susceptibility among slow metabolizers further underscore heterogeneity in risk. Collectively, these findings lend weight to the prioritization of targeted preventive strategies and robust public health policies.

Additional contributions

We gratefully acknowledge the CARES-HAPIN participants and study staff.

Data sharing

Data will be made available on request.

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CRedit authorship contribution statement

Sneha S. Patil: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Andres Cardenas:** Writing – review & editing, Supervision, Conceptualization. **Ajay Pillariseti:** Writing – review & editing, Formal analysis. **Kyle Steenland:** Writing – review & editing, Formal analysis. **Smita S. Patil:** Writing – review & editing, Project administration, Data curation. **Siva Kumar Nuvvula:** Writing – review & editing, Resources, Investigation. **Akash Navilebasappa:** Methodology. **Santu Ghosh:** Writing – review & editing. **Kalpna Balakrishnan:** Writing – review & editing. **Lance A. Waller:** Writing – review & editing, Data curation. **Shirin Jabbarzadeh:** Writing – review & editing, Data curation. **Jennifer Peel:** Writing – review & editing, Funding acquisition. **Thomas F. Clasen:** Writing – review & editing, Investigation, Funding acquisition. **Dana Boyd Barr:** Writing – review & editing, Supervision, Methodology, Investigation. **Naveen Puttaswamy:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jdent.2026.106743](https://doi.org/10.1016/j.jdent.2026.106743).

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