

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

Supplementary Methods

Study Power

No independent sample size calculation was performed for this nested analysis. For context, approximately 578 children would be required to ascertain an ECC prevalence of 40.6% [1] at 95% confidence with a 4% margin of error. Similarly, quantifying ETS exposure prevalence of 52.7% [2] under the same parameters would require 601 children. Detecting subgroup differences in ETS exposure ($\alpha = 0.05$, power = 80%) would require 248 children (124 per group). With 735 children included, the analytical sample exceeded all thresholds for prevalence estimation and subgroup contrasts.

To evaluate the statistical power for the primary associations, we referenced effect sizes reported in prior ETS–ECC studies, which typically fall in the small to moderate range, with odds ratios and risk ratios of about 1.3 to 2.0. Power calculations based on these metrics indicated that the available sample provided roughly 80% power to detect odds ratios of at least 1.4, which is consistent with the magnitude of effects commonly observed in the literature. While some subgroup estimates had wider confidence intervals due to smaller cell sizes, the overall sample was adequate for the main exposure–response analyses.

References: 1. J.A. Henry, M.S. Muthu, A. Saikia, et al. Prevalence and pattern of early childhood caries in a rural South Indian population evaluated by ICDAS with suggestions for enhancement of ICDAS software tool. *Int. J. Paediatr. Dent.*, 27 (3) (2017), pp. 191-200.

2. B.K. Verma, M. Verma, M. Mondal, et al. Alarming trend in under-five Indian children's exposure to indoor tobacco smoke. *Cureus*, 15 (4) (2023), p. e37571.

Data Collection and Quality Assurance

Electronic Data Capture: Data were collected using standardized case report forms (CRFs) implemented in the REDCap mobile platform. Over 80 harmonized CRFs captured sociodemographic, health, nutrition, and environmental exposure data, with contextual adaptations for local languages and settings. Built-in validation rules and skip logic were applied in REDCap to minimize entry errors and ensure data consistency across field sites.

Training and Standardization: Study staff received hands-on training for CRF administration, supported by training videos and detailed standard operating procedures (SOPs). Materials were provided in English and

translated into local languages, followed by a full field “dry run” to ensure consistent administration. SOPs outlined each step from data entry to upload, ensuring uniform procedures across sites and data collectors. Reference: S. Jabbarzadeh, L.M. Jaacks, A. Lovvorn, et al. Data management plan and REDCap mobile data capture for a multi-country Household Air Pollution Intervention Network (HAPIN) trial. *Digit. Health*, 10 (2024), p. 20552076241274217.

Calibration and Reliability

The dental examiner was calibrated prior to data collection. Intra- and inter-examiner reliability testing on 15 children (excluded from the main study) demonstrated sensitivity of 91%–93% and specificity of 98%, and kappa values ranging from 0.88 to 0.92.

The dental examination further recorded missing teeth due to caries, developmental enamel defects, plaque index (Silness and Loe, 1964), consequences of untreated caries (pufa index), skeletal and occlusal characteristics, and dental trauma.

Questionnaires

Parental and Child Characteristics

Extensive questionnaires were administered via interview at baseline and at each follow-up to collect information from both parents. These were abstracted from the HAPIN database for this study.

Maternal and Child Dietary Assessment

Maternal diet during pregnancy was assessed using the Minimum Dietary Diversity for Women (MDD-W) indicator [1], which is based on 10 food groups. Consumption frequencies were categorized as monthly or less, weekly, or daily over the past 30 days.

Child dietary diversity was evaluated at 3–12 months and again at 36–60 months. At 3 months, all infants were exclusively breastfed as per national guidelines, yielding a Dietary Diversity Score (DDS) of 1 (breast milk only). For 6–12 months, dietary intake was assessed following WHO Infant and Young Child Feeding guidelines [2]. Mothers reported all foods and beverages consumed by the child in the preceding 24 hours. Intake was categorized across eight food groups, each scored as 1 if consumed, and summed to create the DDS (range 0–8). The groups were: (i) breast milk; (ii) grains, roots, and tubers; (iii) legumes and nuts; (iv) flesh foods (meat, fish, poultry, organ meats); (v) eggs; (vi) vitamin A-rich fruits and vegetables; (vii) dairy products (milk, yogurt, cheese); and (viii) other fruits and vegetables. At 36–60 months, dietary diversity was

assessed using seven food groups, in line with FAO recommendations. Information on nutritional supplementation, such as iron and vitamins, was also obtained.

To ensure compatibility across time points, the DDS was categorized as low (<4 groups), medium (4–5 groups), or high (>5 groups).

References: 1. FAO. Minimum dietary diversity for women: a guide to measurement. University of California, Davis, Davis (2016).

2. WHO, UNICEF. Indicators for assessing infant and young child feeding practices: definitions and measurement methods. World Health Organization, Geneva (2021).

SHS exposure categories were derived from self-reported SHS exposure across study visits: “unexposed” (no exposure at any time point within the specified period), “ever exposed” (exposure at ≥ 1 time point), and “always exposed” (exposure at all assessed time points). This classification was specified *a priori* to capture exposure persistence and distinguish intermittent from sustained SHS exposure, thereby providing a more accurate representation of cumulative exposure while attenuating the misclassification inherent in single time point assessments. Biomarker-based exposures, by contrast, were modeled using continuous or quantile-based specifications.

Urine Sample Collection, Processing, Storage, and Analysis

Pregnant women provided urine samples during each trimester, and children at 3, 6, 12, and 36–60 months. Preceding each urine collection, women completed an open-ended 24-hour food diary detailing all foods and beverages consumed, including portion sizes and cooking methods. These procedures ensured temporal alignment between dietary intake and biomarker measurements.

Urine samples were collected via standardized clean-catch protocols. Mothers provided first-morning urine, and children’s samples were obtained using pediatric urine bags, a field-validated and culturally acceptable approach. Immediately after collection, samples were stored in temperature-controlled Credo™ ProMed cooler bags (Pelican BioThermal, USA) to maintain the cold chain during transport to the field laboratories. Specific gravity was measured using a refractometer (PAL-10S, Atago, Japan). Samples were then aliquoted into QR-coded cryovials, logged under a chain-of-custody system, frozen at $-20\text{ }^{\circ}\text{C}$, and subsequently transferred on dry ice to the central laboratory at Sri Ramachandra University for storage at $-80\text{ }^{\circ}\text{C}$. Field and transport blanks were analyzed to monitor contamination. Prior to laboratory analysis, aliquoted tubes were thawed at room temperature under controlled conditions.

Briefly, 200 µL of urine was spiked with 50 µL of an internal standard, followed by the addition of 0.85 mL of cold acetone to precipitate salts, proteins, and enzymes. After refrigeration at 4 °C for 15 minutes, the samples were centrifuged at 4000 rpm for 10 minutes. The resulting supernatant (~380 µL) was transferred to a vial containing 20 µL of methanolic HCl. Acetone was partially evaporated, and 10 µL of the remaining solution was injected into LC-ESI-MS/MS for analysis. All analyses were performed without glucuronidase deconjugation, so metabolite concentrations represented free, unbound compounds. A solvent blank, matrix blank, ten calibrants, and duplicates of low-, medium-, and high-level bench quality control samples were prepared and analyzed concurrently.

Reference: D.B. Barr, N. Puttaswamy, L.M. Jaacks, et al. Design and rationale of the HAPIN biomarker center. *Environ. Health Perspect.*, 128 (4) (2020), p. 047010.

Statistical Modeling: Negative Binomial Hurdle (NBH) Model

This model consists of two parts: (1) a logistic component (“zero part”) targets the probability of a child being caries free (ψ), and (2) a log-linear negative binomial regression (“count part”) models the number of affected surfaces in children with caries (μ).

The NBH model can be written as:

$$\text{logit}(\pi) = \delta_0 + \delta_1 x_i + \delta' w_i$$

$$\log(\mu) = \lambda_0 + \lambda_1 x_i + \lambda' w_i,$$

where δ_0 , δ_1 , λ_0 and λ_1 are unknown parameters, δ' and λ' are unknown parameter vectors, x_i is the secondhand smoke exposure, and w_i is a vector of observed covariate values for individual i with response μ ($i = 1, \dots, n$). The binary part reports odds ratios, and the count estimates incidence rate ratios.

Model selection

The NBH model was chosen for its interpretability and its ability to separately estimate the probability of remaining caries free. Both the NBH and zero-inflated negative binomial models [1] outperformed their Poisson counterparts, as reflected in their lower AIC and BIC values and higher log-likelihood estimates for both prenatal and postnatal analyses. The Vuong test [2] ($w^2 = 0.000$, $p = 0.916$ for prenatal; $w^2 = 0.000$, $p = 0.955$ for postnatal) showed no significant difference between the two negative binomial models, suggesting comparable fit.

	Zero-inflated Poisson	Zero-inflated negative binomial	Hurdle Poisson	Hurdle negative binomial
Average Prenatal				
log likelihood	-3614.21	-2215.7	-3614.21	-2215.71
AIC	7236.47	4441.40	7236.42	4441.41
BIC	7254.69	4464.24	7254.69	4464.25
Vuong test	w2 = 0.000, p = 0.916			
Average Postnatal				
log likelihood	-3645.18	-2206.98	-3645.18	-2206.98
AIC	7298.35	4423.95	7298.35	4423.96
BIC	7316.59	4446.74	7316.59	4446.75
Vuong test	w2 = 0.000, p = 0.955			

AIC = Akaike information criterion; BIC = Bayesian information criterion

References: 1. J. Mullahy. Specification and testing of some modified count data models. J. Econom., 33 (1986), pp. 341-365.

2. Q. Vuong. Likelihood ratio tests for model selection and non-nested hypotheses. Econometrica, 57 (1989), pp. 307-334.

Supplementary Results

Association between covariates and ECC

Table 1 delineates the sociodemographic, perinatal, and behavioral characteristics of the children stratified by caries status. Among the 712 children, the proportions of CF, NCL, and CL were 234 (32.8%), 97 (13.6%), and 381 (53.6%), respectively. The mean age at ECC assessment was 4.0 years, with subtle yet statistically discernible variation across categories ($p = 0.043$). Gender distribution and child's BMI did not differ significantly ($p = 0.580$, $p = 0.651$, respectively). Maternal and paternal education both exhibited significant associations, although higher parental education was unexpectedly more common among NCL and CL children (mother, $p = 0.017$; father, $p < 0.001$). Dietary diversity revealed significant disparities, with low diversity more frequent among CL children and, to a lesser extent, those with NCL ($p = 0.038$). Eighteen percent experienced moderate to severe food insecurity.

Pregnancy and perinatal characteristics such as mode of delivery, preterm birth, and low birth weight did not demonstrate significant variation across groups. In contrast, prenatal SHS exposure was robustly correlated with ECC status, as 39.4% of mothers of CL children reported exposure compared to 32.9% of CF and 27.8% of NCL ($p = 0.001$). Both postnatal and current SHS exposures were elevated among CL children, with intermediate prevalence in the NCL group ($p = 0.013$ and $p = 0.035$, respectively).

Children's dietary and oral hygiene practices further differentiated group differences. Early introduction of sugar (<12 months) was markedly associated with ECC, with exposure reported by 72.4% of CL and 70.1% of NCL compared to only 33.3% of CF children ($p < 0.001$). Frequency of snacking also exhibited pronounced gradients ($p < 0.001$): twice-daily snacking was more common among NCL (43.3%) and CL (32.1%) children than CF (23.1%). Brushing frequency was inversely associated with ECC: twice-daily brushing was disproportionately represented among CF children (76.9%), whereas once-daily brushing predominated in CL (56.9%) and NCL (44.3%) children ($p < 0.001$). Finally, toothpaste use further accentuated intergroup disparities ($p < 0.001$): fluoridated toothpaste use was highest in CL (60.9%), intermediate in CF (42.3%), and lowest in NCL (32.9%) children. This may reflect reverse causation, with parents adopting preventive measures after disease onset.

Appendix Table 1. Summary of quality control measurements for urinary nicotine biomarkers (CARES - HAPIN, 2023-2025, Tamil Nadu, India)

Analyte	Total N ¹	Detection rate (%) ²	LOD ng/mL	Recovery (%) ³ mean (SD)	Precision (CV) min-max
Cotinine	3973	97.6	0.05	81.7 (6.4)	0.62 - 4.65
3-hydroxycotinine	3973	98.4	0.05	88.7 (6.8)	0.65 - 5.49

¹Total urine samples analyzed across all time points; ²Samples with levels above detection limit; ³Recovery of spiked stable isotope of cotinine-*d3* and, 3-hydroxycotinine-*d3*. LOD – limit of detection

Appendix Table 2: Summary of urine samples analyzed and proportion below limit of detection at each sampling time point

Timepoint	Total Samples (n)	Below LOD, n (%)	
		Cotinine	3-hydroxycotinine
First Trimester	711	47 (6.6%)	27 (3.8%)
Second Trimester	684	48 (7.0%)	18 (2.6%)
Third Trimester	608	33 (5.4%)	20 (3.3%)
Three months	595	35 (5.9%)	13 (2.2%)
Six months	604	35 (5.8%)	37 (6.1%)
Twelve months	598	23 (3.8%)	31 (5.2%)
36–60 months	172	0	0

Appendix Table 3. Geometric means of cotinine, 3-hydroxycotinine, and nicotine equivalents (µg/g creatinine; 95% CI) among CARES-HAPIN children by demographic groups (2023-2025, Tamil Nadu, India)

Characteristic	N	Cotinine		3-hydroxycotinine		Nicotine Equivalents (NE2)	
		Prenatal	Postnatal	Prenatal	Postnatal	Prenatal	Postnatal
Overall	712	1.65 (1.47, 1.82)	5.21 (5.03, 5.40)	4.32 (4.16, 4.48)	9.49 (9.33, 9.65)	6.32 (6.17, 6.47)	15.87 (15.72, 16.02)
Gender							
Boy	380	1.65 (1.41, 1.89)	5.26 (5.01, 5.51)	4.36 (4.14, 4.58)	9.65 (9.44, 9.86)	6.41 (6.20, 6.61)	16.07 (15.88, 16.27)
Girl	332	1.64 (1.39, 1.90)	5.12 (4.85, 5.40)	4.27 (4.04, 4.50)	9.27 (9.04, 9.51)	6.23 (6.01, 6.45)	15.56 (15.34, 15.78)
Mother's education							
No formal education	255	1.60 (1.35, 1.84)	4.95 (4.65, 5.24)	4.13 (3.96, 4.29)	8.50 (8.24, 8.76)	6.08 (5.93, 6.23)	14.59 (14.35, 14.82)
Primary school completed	198	1.69 (1.37, 2.01)	4.90 (4.52, 5.28)	4.48 (4.19, 4.77)	10.09 (9.79, 10.38)	6.52 (6.25, 6.80)	16.04 (15.75, 16.33)
Secondary school or college completed	259	1.67 (1.37, 1.97)	5.70 (5.40, 6.00)	4.39 (4.13, 4.65)	10.02 (9.76, 10.29)	6.41 (6.17, 6.66)	16.97 (16.73, 17.21)
Father's education							
No formal education	276	1.59 (1.30, 1.88)	5.26 (4.98, 5.55)	4.19 (3.93, 4.46)	9.38 (9.13, 9.62)	6.17 (5.92, 6.41)	15.74 (15.51, 15.96)
Primary school completed	222	1.75 (1.44, 2.05)	5.05 (4.71, 5.38)	4.35 (4.07, 4.63)	9.31 (9.03, 9.59)	6.44 (6.18, 6.71)	15.54 (15.28, 15.80)
Secondary school or college completed	213	1.62 (1.29, 1.95)	5.32 (4.97, 5.67)	4.46 (4.16, 4.75)	9.83 (9.53, 10.14)	6.40 (6.12, 6.68)	16.39 (16.11, 16.67)
Dietary diversity							
Low	558	1.65 (1.46, 1.84)	5.09 (4.88, 5.30)	4.35 (4.18, 4.53)	9.39 (9.21, 9.57)	6.33 (6.16, 6.49)	15.67 (15.50, 15.83)
Medium	131	1.65 (1.18, 2.13)	5.69 (5.28, 6.11)	4.22 (3.82, 4.61)	9.96 (9.61, 10.30)	6.32 (5.95, 6.70)	16.75 (16.43, 17.08)

High	23	1.60 (0.54, 2.65)	5.65 (4.56, 6.73)	4.05 (3.19, 4.91)	9.24 (8.38, 10.10)	6.16 (5.34, 6.98)	15.90 (15.05, 16.75)
Low birthweight							
Yes	283	1.64 (1.36, 1.92)	5.67 (5.39, 5.95)	4.31 (4.05, 4.56)	9.22 (8.97, 9.47)	6.33 (6.09, 6.56)	15.94 (15.71, 16.17)
No	429	1.65 (1.43, 1.87)	4.93 (4.68, 5.18)	4.33 (4.12, 4.53)	9.69 (9.48, 9.89)	6.32 (6.13, 6.51)	15.84 (15.65, 16.03)
Self-reported secondhand smoke exposure							
Unexposed	337	1.58 (1.33, 1.84)	5.48 (5.22, 5.74)	4.25 (4.01, 4.49)	9.84 (9.62, 10.06)	6.17 (5.94, 6.39)	16.44 (16.23, 16.65)
Ever-exposed	271	1.66 (1.39, 1.93)	4.91 (4.59, 5.23)	4.35 (4.10, 4.60)	9.11 (8.85, 9.37)	6.36 (6.12, 6.60)	15.18 (14.94, 15.43)
Always-exposed	104	1.84 (1.36, 2.31)	5.16 (4.70, 5.62)	4.46 (4.08, 4.84)	9.52 (9.08, 9.96)	6.75 (6.38, 7.12)	15.94 (15.55, 16.33)
ECC stages							
No caries	234	1.48 (1.17, 1.79)	4.46 (4.10, 4.82)	3.90 (3.62, 4.18)	8.50 (8.22, 8.79)	5.76 (5.51, 6.02)	14.15 (13.88, 14.42)
Non-cavitated lesions	97	1.63 (1.21, 2.05)	5.42 (4.92, 5.92)	4.45 (4.05, 4.85)	9.77 (9.32, 10.22)	6.40 (6.02, 6.77)	16.38 (15.96, 16.79)
Cavitated lesions	381	1.77 (1.52, 2.01)	5.73 (5.49, 5.96)	4.56 (4.34, 4.78)	10.09 (9.88, 10.30)	6.67 (6.46, 6.88)	16.94 (16.75, 17.13)

Appendix Table 4. Primary analyses: negative binomial hurdle model estimates for longitudinal urinary nicotine biomarkers and ECC risk among CARES-HAPIN children (2023–2025, Tamil Nadu, India)

Timepoint	N	Count part			Zero part		
		Coefficient	IRR (95% CI)	p-value	Coefficient	OR (95% CI)	p-value
Cotinine							
First Trimester ^a	711	0.09	1.10 (1.05–1.15)	<0.001	0.06	1.06 (0.93–1.20)	0.370
Second Trimester ^a	684	0.05	1.05 (0.99–1.12)	0.073	0.16	1.18 (1.02–1.36)	0.023
Third Trimester ^a	608	0.07	1.08 (1.01–1.14)	0.022	-0.09	0.92 (0.79–1.06)	0.240
Three months ^a	595	0.05	1.05 (0.98–1.12)	0.164	0.07	1.07 (0.90–1.27)	0.435
Six months ^a	604	0.08	1.08 (1.02–1.15)	0.013	0.15	1.17 (1.01–1.35)	0.043
Twelve months ^a	598	0.09	1.09 (1.03–1.16)	0.003	0.04	1.04 (0.91–1.19)	0.547
36–60 months ^a	172	0.14	1.15 (0.98–1.36)	0.083	0.41	1.51 (0.86–2.64)	0.149
Average Prenatal ^a	712	0.19	1.20 (1.12–1.30)	<0.001	0.12	1.13 (0.92–1.38)	0.238
Average Postnatal ^b	705	0.15	1.16 (1.08–1.25)	<0.001	0.29	1.34 (1.12–1.61)	0.002
3-hydroxycotinine							
First Trimester ^a	711	0.09	1.10 (1.04–1.16)	<0.001	0.06	1.06 (0.93–1.21)	0.368
Second Trimester ^a	684	0.08	1.09 (1.02–1.15)	0.006	0.12	1.13 (0.98–1.30)	0.090
Third Trimester ^a	608	0.09	1.10 (1.03–1.17)	0.005	-0.01	0.99 (0.86–1.14)	0.875
Three months ^a	595	-0.01	0.99 (0.93–1.05)	0.706	-0.01	0.99 (0.84–1.17)	0.913
Six months ^a	604	0.05	1.05 (0.99–1.11)	0.128	0.15	1.16 (1.01–1.33)	0.034
Twelve months ^a	598	-0.04	0.96 (0.91–1.02)	0.183	0.05	1.05 (0.92–1.20)	0.493
36–60 months ^a	171	0.03	1.03 (0.92–1.15)	0.595	-0.10	0.90 (0.62–1.31)	0.586
Average Prenatal ^a	712	0.22	1.24 (1.14–1.36)	<0.001	0.20	1.22 (0.98–1.53)	0.081
Average Postnatal ^b	705	0.13	1.13 (1.04–1.24)	0.006	0.36	1.43 (1.16–1.76)	0.001
Nicotine Equivalents (NE2; cotinine + 3-hydroxycotinine)							
First Trimester ^a	711	0.12	1.13 (1.07–1.20)	<0.001	0.07	1.07 (0.92–1.24)	0.380
Second Trimester ^a	684	0.11	1.11 (1.04–1.19)	0.003	0.15	1.16 (0.99–1.37)	0.074

Third Trimester ^a	608	0.11	1.12 (1.04–1.20)	0.003	-0.07	0.93 (0.79–1.10)	0.415
Three months ^a	595	0.02	1.02 (0.94–1.10)	0.691	-0.02	0.98 (0.80–1.20)	0.874
Six months ^a	604	0.07	1.07 (1.00–1.14)	0.060	0.18	1.20 (1.02–1.41)	0.030
Twelve months ^a	598	0.06	1.06 (0.98–1.14)	0.130	0.06	1.06 (0.90–1.25)	0.483
36–60 months ^a	172	0.08	1.09 (0.94–1.26)	0.267	-0.11	0.90 (0.54–1.49)	0.679
Average Prenatal ^a	712	0.26	1.29 (1.18–1.42)	<0.001	0.18	1.20 (0.94–1.53)	0.150
Average Postnatal ^b	705	0.17	1.19 (1.08–1.31)	0.001	0.39	1.47 (1.16–1.86)	0.001

^aAdjusted 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

^bAdditionally adjusted for average prenatal levels

Appendix Table 5. Sensitivity analyses (restricted covariates): negative binomial hurdle model estimates for longitudinal urinary nicotine biomarkers and ECC risk among CARES-HAPIN children (2023–2025, Tamil Nadu, India)

Timepoint	N	Count part			Zero part		
		Coefficient	IRR (95% CI)	p-value	Coefficient	OR (95% CI)	p-value
Cotinine							
First Trimester ^a	711	0.09	1.10 (1.05–1.15)	<0.001	0.10	1.10 (0.98–1.24)	0.091
Second Trimester ^a	684	0.06	1.06 (1.00–1.12)	0.061	0.18	1.20 (1.06–1.37)	0.005
Third Trimester ^a	608	0.07	1.07 (1.01–1.14)	0.027	-0.02	0.98 (0.86–1.12)	0.784
Three months ^a	595	0.04	1.04 (0.97–1.11)	0.285	0.03	1.04 (0.89–1.20)	0.651
Six months ^a	604	0.08	1.09 (1.02–1.16)	0.008	0.19	1.20 (1.05–1.38)	0.007
Twelve months ^a	598	0.10	1.10 (1.04–1.17)	0.001	0.12	1.12 (1.00–1.27)	0.053
36–60 months ^a	172	0.15	1.16 (0.99–1.36)	0.058	0.36	1.44 (0.90–2.28)	0.125
Average Prenatal ^a	712	0.19	1.21 (1.12–1.31)	<0.001	0.22	1.24 (1.04–1.49)	0.018
Average Postnatal ^b	705	0.14	1.15 (1.07–1.24)	<0.001	0.31	1.36 (1.16–1.60)	<0.001
3-hydroxycotinine							

First Trimester ^a	711	0.09	1.10 (1.04–1.16)	<0.001	0.09	1.10 (0.98–1.23)	0.111
Second Trimester ^a	684	0.09	1.09 (1.03–1.16)	0.003	0.13	1.14 (1.01–1.30)	0.041
Third Trimester ^a	608	0.09	1.09 (1.03–1.17)	0.006	0.07	1.07 (0.94–1.22)	0.305
Three months ^a	595	-0.01	0.99 (0.93–1.06)	0.733	0.03	1.03 (0.89–1.20)	0.682
Six months ^a	604	0.05	1.05 (0.99–1.12)	0.088	0.16	1.18 (1.04–1.33)	0.009
Twelve months ^a	598	-0.03	0.97 (0.92–1.02)	0.259	0.09	1.10 (0.97–1.24)	0.129
36–60 months ^a	171	0.04	1.04 (0.93–1.16)	0.493	-0.11	0.90 (0.66–1.23)	0.499
Average Prenatal ^a	712	0.22	1.25 (1.14–1.36)	<0.001	0.27	1.31 (1.07–1.60)	0.010
Average Postnatal ^b	705	0.13	1.14 (1.04–1.24)	0.004	0.35	1.42 (1.18–1.72)	<0.001
Nicotine Equivalents (NE2; cotinine + 3-hydroxycotinine)							
First Trimester ^a	711	0.13	1.13 (1.07–1.20)	<0.001	0.12	1.13 (0.99–1.29)	0.064
Second Trimester ^a	684	0.11	1.12 (1.05–1.20)	0.001	0.18	1.19 (1.03–1.38)	0.020
Third Trimester ^a	608	0.11	1.11 (1.04–1.20)	0.004	0.02	1.02 (0.88–1.19)	0.776
Three months ^a	595	0.01	1.01 (0.94–1.10)	0.730	0.02	1.02 (0.85–1.22)	0.837
Six months ^a	604	0.07	1.08 (1.01–1.15)	0.034	0.21	1.23 (1.06–1.43)	0.006
Twelve months ^a	598	0.07	1.07 (1.00–1.16)	0.061	0.15	1.16 (1.00–1.35)	0.044
36–60 months ^a	172	0.10	1.10 (0.95–1.27)	0.195	-0.10	0.90 (0.59–1.37)	0.635
Average Prenatal ^a	712	0.26	1.30 (1.19–1.43)	<0.001	0.28	1.33 (1.06–1.65)	0.012
Average Postnatal ^b	705	0.17	1.19 (1.08–1.31)	<0.001	0.42	1.52 (1.23–1.88)	<0.001

^aAdjusted for socioeconomic status index, child's age at dental evaluation, gender, and body mass index

^bAdditionally adjusted for average prenatal levels

Appendix Table 6. Sensitivity analyses (expanded covariates): negative binomial hurdle model estimates for longitudinal urinary nicotine biomarkers and ECC risk among CARES-HAPIN children (2023–2025, Tamil Nadu, India)

Timepoint	N	Count part			Zero part		
		Coefficient	IRR (95% CI)	p-value	Coefficient	OR (95% CI)	p-value
Cotinine							
First Trimester ^a	711	0.09	1.09 (1.03–1.15)	0.001	0.08	1.09 (0.95–1.24)	0.212
Second Trimester ^a	684	0.06	1.06 (1.00–1.13)	0.039	0.17	1.18 (1.03–1.37)	0.020
Third Trimester ^a	608	0.07	1.07 (1.00–1.14)	0.037	-0.09	0.91 (0.79–1.06)	0.234
Three months ^a	595	0.04	1.04 (0.97–1.12)	0.234	0.06	1.07 (0.90–1.26)	0.470
Six months ^a	604	0.08	1.08 (1.02–1.15)	0.014	0.15	1.16 (1.00–1.36)	0.054
Twelve months ^a	598	0.07	1.07 (1.01–1.14)	0.026	0.06	1.06 (0.93–1.22)	0.391
36–60 months ^a	172	0.15	1.16 (0.99–1.37)	0.072	0.34	1.40 (0.79–2.49)	0.246
Average Prenatal ^a	712	0.18	1.20 (1.10–1.30)	<0.001	0.15	1.16 (0.95–1.43)	0.154
Average Postnatal ^b	705	0.14	1.15 (1.06–1.24)	0.001	0.29	1.34 (1.11–1.62)	0.002
3-hydroxycotinine							
First Trimester ^a	711	0.09	1.09 (1.04–1.15)	0.001	0.08	1.08 (0.95–1.23)	0.251
Second Trimester ^a	684	0.09	1.10 (1.03–1.17)	0.002	0.12	1.13 (0.98–1.30)	0.104
Third Trimester ^a	608	0.09	1.10 (1.03–1.17)	0.005	-0.02	0.98 (0.85–1.13)	0.784
Three months ^a	595	-0.01	0.99 (0.93–1.05)	0.691	-0.01	0.99 (0.83–1.18)	0.929
Six months ^a	604	0.04	1.04 (0.98–1.10)	0.174	0.14	1.16 (1.01–1.33)	0.040
Twelve months ^a	598	-0.04	0.96 (0.91–1.02)	0.156	0.05	1.05 (0.91–1.20)	0.493
36–60 months ^a	171	0.03	1.03 (0.92–1.16)	0.567	-0.18	0.83 (0.56–1.24)	0.370
Average Prenatal ^a	712	0.22	1.25 (1.14–1.37)	<0.001	0.21	1.23 (0.98–1.55)	0.071
Average Postnatal ^b	705	0.12	1.13 (1.03–1.23)	0.011	0.36	1.43 (1.16–1.77)	0.001
Nicotine Equivalents (NE2; cotinine + 3-hydroxycotinine)							
First Trimester ^a	711	0.12	1.13 (1.06–1.20)	<0.001	0.09	1.10 (0.94–1.28)	0.232
Second Trimester ^a	684	0.12	1.12 (1.05–1.20)	0.001	0.15	1.16 (0.98–1.37)	0.081

Third Trimester ^a	608	0.11	1.11 (1.03–1.20)	0.004	-0.08	0.92 (0.78–1.10)	0.364
Three months ^a	595	0.01	1.01 (0.94–1.09)	0.772	-0.02	0.98 (0.80–1.20)	0.845
Six months ^a	604	0.06	1.06 (0.99–1.14)	0.080	0.18	1.19 (1.01–1.41)	0.037
Twelve months ^a	598	0.06	1.04 (0.97–1.12)	0.292	0.07	1.07 (0.91–1.27)	0.411
36–60 months ^a	172	0.09	1.10 (0.94–1.28)	0.243	-0.21	0.81 (0.47–1.40)	0.458
Average Prenatal ^a	712	0.26	1.29 (1.17–1.42)	<0.001	0.20	1.22 (0.95–1.57)	0.121
Average Postnatal ^b	705	0.16	1.17 (1.06–1.29)	0.002	0.39	1.48 (1.16–1.88)	0.001

^aAdjusted for socioeconomic status index, father's education, child's age at dental evaluation, gender, body mass index, timing of first sugar intake, frequency of snacking, use of fluoridated toothpaste and feeding after brushing

^bAdditionally adjusted for average prenatal levels

Appendix Table 7. Influence of smoking habits on ECC prevalence from the prenatal period to early childhood among CARES-HAPIN children (2023-2025, Tamil Nadu, India)

	Prevalence	OR (95% CI)	p-value
Smokers in home			
One	174/280 (62.1%)	0.88 (0.64, 1.22)	0.232
Two	85/95 (89.4%)	4.57 (2.29, 9.15)	<0.001
Daily cigarette consumption (number of cigarettes)			
1 to 10	199/298 (66.7%)	1.08 (0.77, 1.50)	0.317
≥11	60/77 (77.9%)	1.90 (1.10, 3.41)	0.015

Appendix Table 8. Additive effect of secondhand smoke exposure on ECC prevalence during prenatal and postnatal periods among CARES-HAPIN children (2023-2025, Tamil Nadu, India)

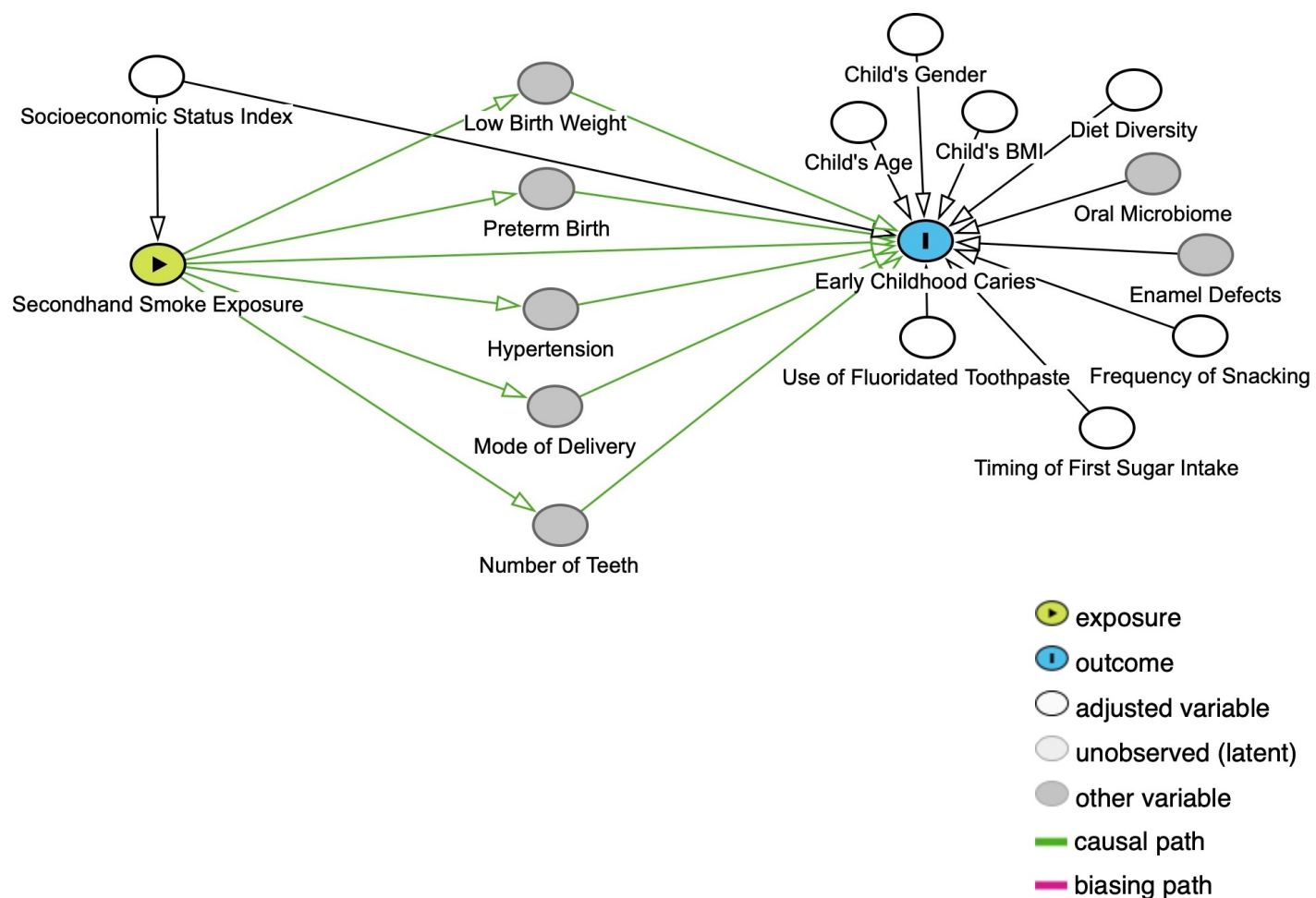
Time period		Prevalence	OR (95% CI)	p-value
Prenatal SHS exposure	Postnatal SHS exposure			
No	No	272/414 (65.7%)	1.00	
Yes	No	9/21 (42.8%)	0.91 (0.43, 1.91)	0.405
No	Yes	29/44 (65.9%)	1.00 (0.52, 1.94)	0.488
Yes	Yes	170/233 (72.9%)	1.35 (1.00, 2.05)	0.028

Appendix Table 9. Adjusted odds ratio (95% CI) for the nicotine metabolizing ratio at various time points in CARES-HAPIN children (2023-2025, Tamil Nadu, India)

	N	Coefficient	OR (95% CI)	p-value
Slow metabolizers				
First Trimester	178	-0.02	0.98 (0.69, 1.39)	0.902
Second Trimester	172	0.05	1.05 (0.75, 1.49)	0.763
Third Trimester	152	0.55	1.74 (1.15, 2.64)	0.009
Three months	149	0.25	1.29 (0.84, 1.97)	0.242
Six months	151	0.16	1.17 (0.75, 1.83)	0.492
Twelve months	150	-0.07	0.94 (0.64, 1.38)	0.736
36–60 months	43	-0.47	0.62 (0.17, 2.32)	0.481
Average Prenatal	178	0.58	1.79 (0.94, 3.40)	0.077
Average Postnatal	177	0.30	1.35 (0.82, 2.24)	0.242
Fast metabolizers				
First Trimester	533	0.04	1.05 (0.85, 1.29)	0.679
Second Trimester	513	-0.22	0.80 (0.63, 1.01)	0.06
Third Trimester	456	-0.03	0.98 (0.77, 1.23)	0.803
Three months	446	-0.10	0.91 (0.70, 1.17)	0.465
Six months	452	-0.19	0.83 (0.68, 1.02)	0.076
Twelve months	448	-0.04	0.96 (0.79, 1.17)	0.701
36–60 months	128	-0.41	0.65 (0.45, 1.00)	0.045
Average Prenatal	534	-0.25	0.78 (0.54, 1.13)	0.184
Average Postnatal	528	-0.27	0.75 (0.57, 1.02)	0.063

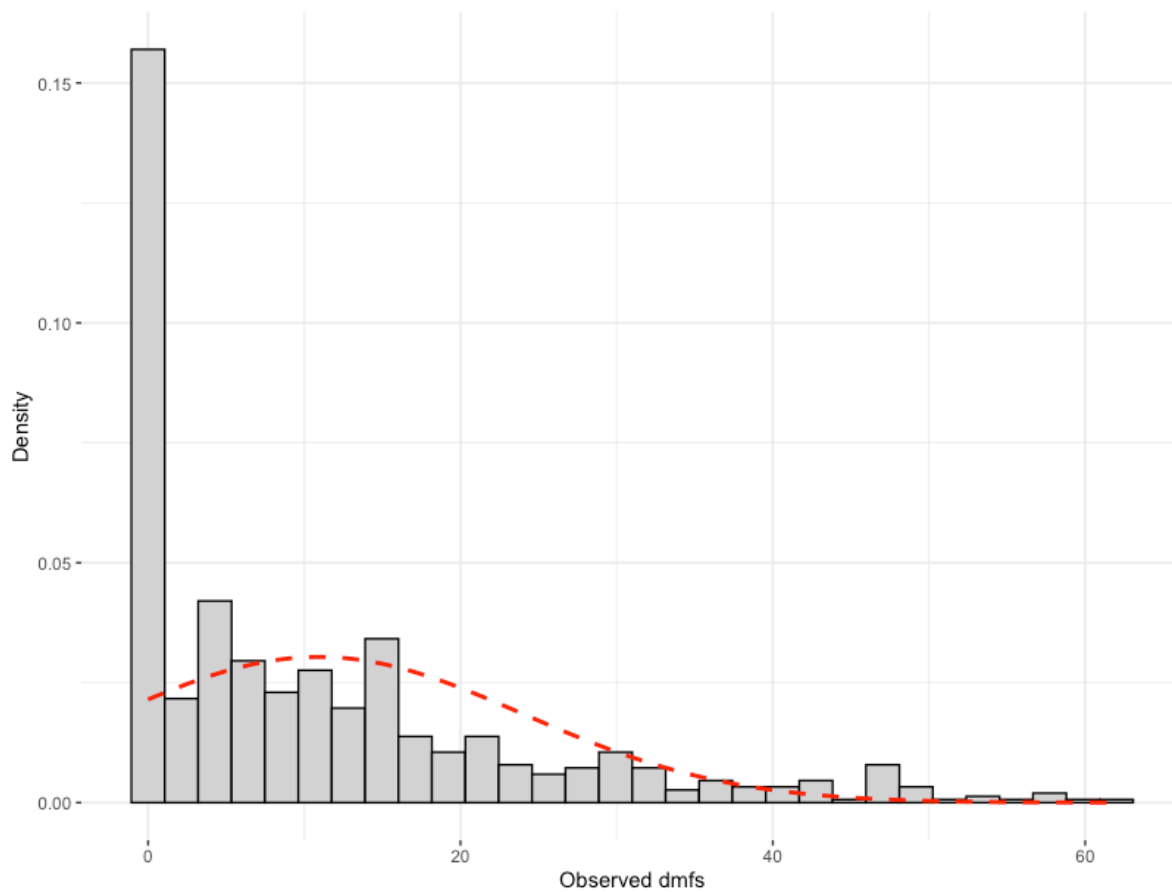
Adjusted for socioeconomic status index, child's age at dental evaluation, gender, and body mass index

Appendix Figure 1. Directed acyclic graph for the proposed association between secondhand smoke exposure and childhood caries experience



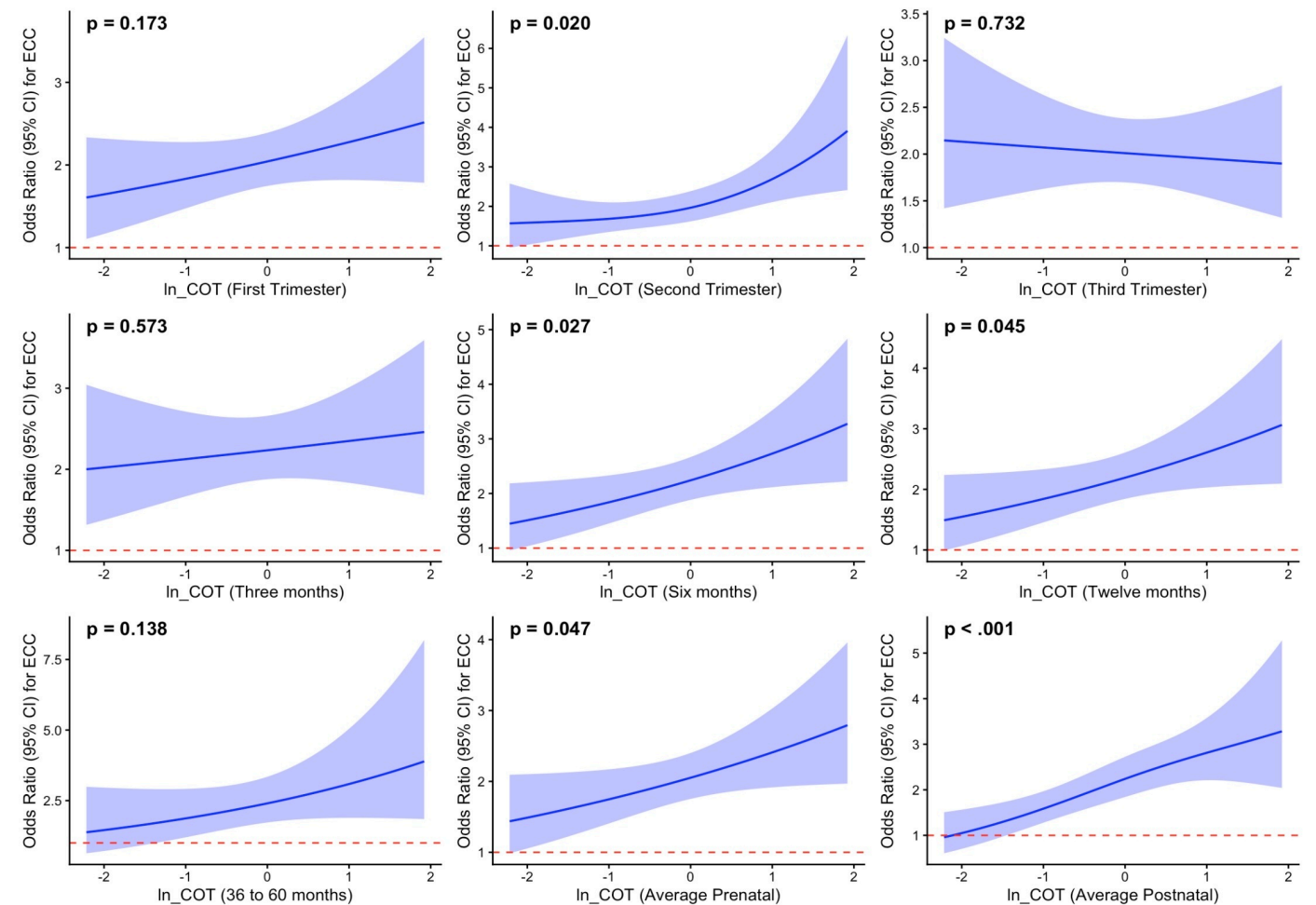
Appendix Figure 2: Distribution of dmfs scores among CARES-HAPIN children (n=712)

*The red line depicts normal distribution fit



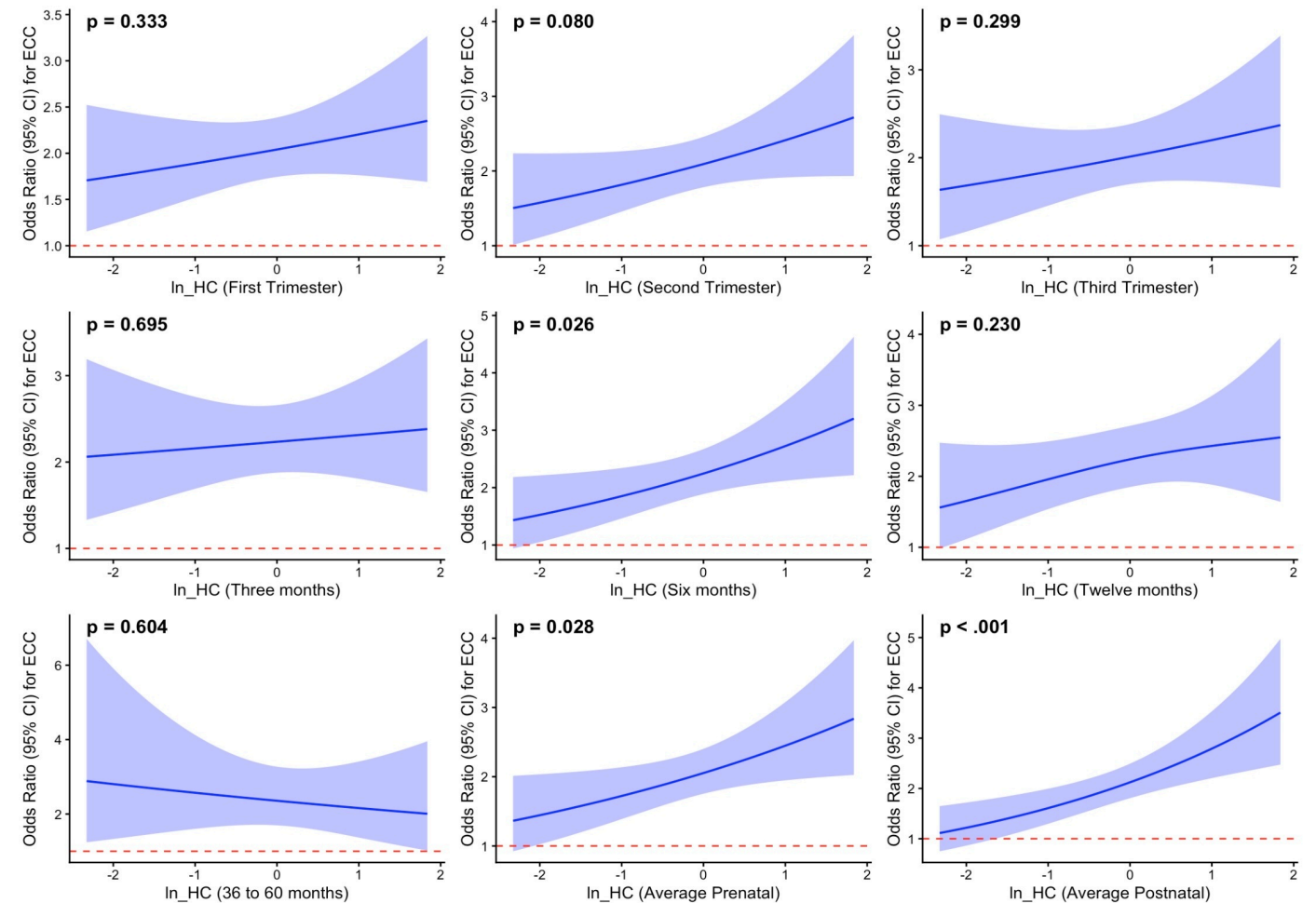
Appendix Figure 3. Exposure-response relationships between cotinine concentrations and ECC risk across time points among CARES-HAPIN children

*Solid line represents predicted probability of caries; shaded area indicates 95% confidence interval



Appendix Figure 4. Exposure-response relationships between 3-hydroxycotinine concentrations and ECC risk across time points among CARES-HAPIN children

*Solid line represents predicted probability of caries; shaded area indicates 95% confidence interval



Appendix Figure 5. Exposure-response relationships between total secondhand smoke (Nicotine Equivalents (NE2)) exposure concentrations and ECC risk across time points among CARES-HAPIN children

*Solid line represents predicted probability of caries; shaded area indicates 95% confidence interval

