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Differential Associations of Hand Nicotine and Urinary Cotinine with Children's Exposure to Tobacco Smoke and Clinical Outcomes

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ABSTRACT

Background: Children's overall tobacco smoke exposure (TSE) consists of both inhalation of secondhand smoke (SHS) and ingestion, dermal uptake, and inhalation of thirdhand smoke (THS) residue from dust and surfaces in their environments.

Objectives: Our objective was to compare the different roles of urinary cotinine as a biomarker of recent overall TSE and hand nicotine as a marker of children's contact with nicotine pollution in their environments. We explored the differential associations of these markers with sociodemographics, parental smoking, child TSE, and clinical diagnoses.

Methods: Data were collected from 276 pediatric emergency department patients (Median age=4.0 years) who lived with a cigarette smoker. Children's hand nicotine and urinary cotinine levels were determined using LC-MS/MS. Parents reported tobacco use and child TSE. Medical records were reviewed to assess discharge diagnoses.

Results: All children had detectable hand nicotine (GeoM=89.7ng/wipe; 95% CI=[78.9;102.0]) and detectable urinary cotinine (GeoM=10.4ng/ml; 95%CI=[8.5;12.6]). Although urinary cotinine and hand nicotine were highly correlated (r=0.62, p<0.001), urinary cotinine geometric means differed between racial groups and were higher for children with lower family income (p<0.05), unlike hand nicotine. Independent of urinary cotinine, age, race, and ethnicity, children with higher hand nicotine levels were at increased risk to have discharge diagnoses of viral/other infectious illness (aOR=7.49; 95%CI=[2.06;27.24], *p*=0.002), pulmonary illness (aOR=6.56; 95%CI=[1.76;24.43], *p*=0.005), and bacterial infection (aOR=5.45; 95%CI=[1.50;19.85], *p*=0.03). In contrast, urinary cotinine levels showed no associations with diagnosis independent of child hand nicotine levels and demographics.

Discussion: The differential associations of hand nicotine and urinary cotinine suggest the two markers reflect different exposure profiles that contribute differentially to pediatric illness. Because THS in a child's environment directly contributes to hand nicotine, additional studies of children of smokers and nonsmokers are warranted to determine the role of hand nicotine as a marker of THS exposure and its potential role in the development of tobacco-related pediatric illnesses.

KEYWORDS

Thirdhand smoke, secondhand smoke, environmental tobacco smoke, tobacco smoke pollution, cotinine.

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Introduction

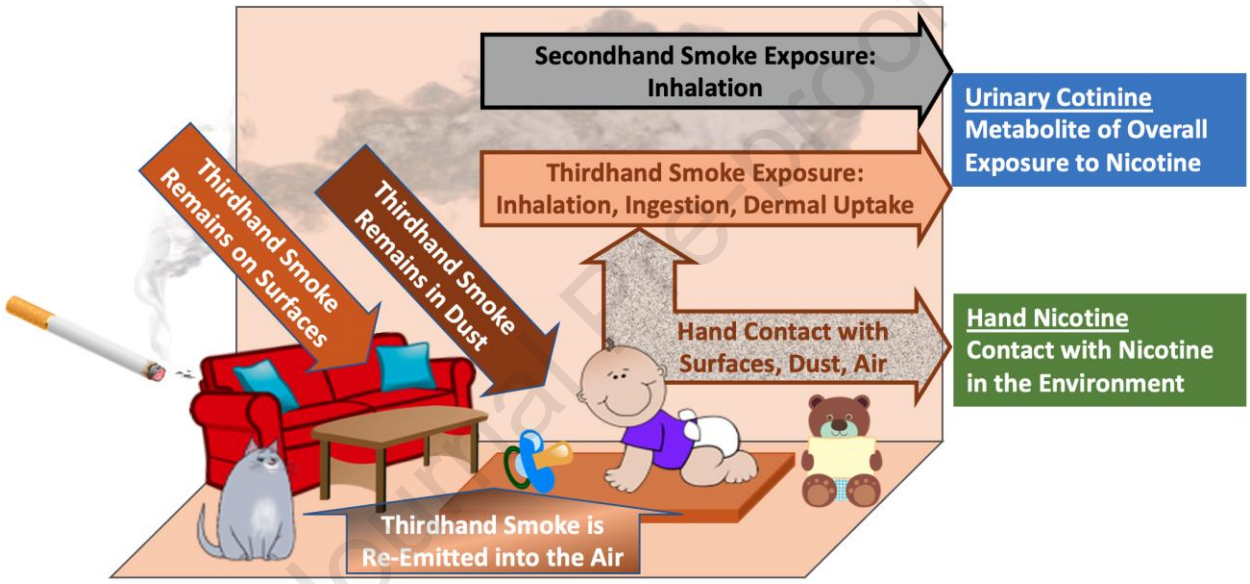
Children may be exposed to tobacco smoke pollutants by inhaling freshly-emitted secondhand smoke (SHS) and also via dermal uptake, ingestion, and inhalation of tobacco smoke residue present on surfaces and in dust, also known as thirdhand smoke (THS) (Jacob et al., 2017; Matt et al., 2011a; Merianos et al., 2019). Cotinine, a metabolite of nicotine (Benowitz et al., 2009), is the biomarker most commonly used to measure overall tobacco smoke exposure (TSE) from SHS and THS. Because cotinine cannot differentiate between SHS and THS exposure and has a relatively short half-life (16-20 hours) (Benowitz et al., 2009), it is of limited use to measure exposure to chronic THS pollution in children who may also be exposed to SHS. Since nicotine is mainly metabolized in the liver, cotinine levels are influenced by several biological processes unrelated to TSE. Most notably, this includes genetic differences in the frequency of *CYP2A6* variant alleles, which affect the rate of nicotine metabolism; these differences have been causally linked to differences in cotinine levels between children of different sex and racial backgrounds (Benowitz et al., 2006; Dempsey et al., 2013; Zhu et al., 2013).

To address some of the limitations of cotinine, hand nicotine has been proposed as a marker of THS pollution with which children come in contact in their environment (Kelley et al., 2021; Mahabee-Gittens et al., 2021; Mahabee-Gittens et al., 2019; Mahabee-Gittens et al., 2018). Unlike cotinine, hand nicotine is not affected by metabolic processes and provides a more proximal indicator of THS pollutants in the dust and on surfaces a child's hand may have touched. Hand nicotine may be a particularly useful measure for young children because of their frequent hand-to-mouth activities, mouthing behavior (e.g., sucking, chewing on objects), and mouthing behavior (i.e., ingestion of non-food items) (Xue et al., 2007). A recent study by Diamond et al., (Diamond et al., 2021) demonstrated the prominent role of hand-based transport of semi-volatile chemicals (SVOC) in adults from surfaces in the indoor environment. Other work indicates that skin serves as a nicotine reservoir and that nicotine can be dermally absorbed from the air, clothes, and organic matter (e.g., tobacco leaves) (Beko et al., 2017; Curwin et al., 2005; Frasch and Barbero, 2017). Pilot research conducted by our group has shown that nicotine (an SVOC) on hands was highest in children who are 2-4 years old and who live in homes where more cigarettes are smoked (Mahabee-Gittens et al., 2019; Mahabee-Gittens et al., 2018). This preliminary research demonstrates that hand nicotine and cotinine levels provide related but nonredundant measures of TSE to both SHS and THS (Mahabee-Gittens et al., 2018).

Figure 1 illustrates the contribution of SHS and THS to cotinine and hand nicotine measures. SHS exposure occurs exclusively via inhalation of exhaled main-stream and side-

stream smoke. THS exposure occurs through dermal uptake, ingestion, and inhalation of tobacco smoke residue that lingers on surfaces, accumulates in dust, is embedded in objects, and is re-emitted from THS reservoirs into the air. We propose that measures of cotinine and hand nicotine represent different exposure pathways and exposure profiles. Further, we hypothesize that hand nicotine may provide insights into children's exposure-relevant behaviors and interaction with chronic THS pollution in their homes that differ from overall TSE indicated by cotinine.

Figure 1: Multiple exposure pathways and markers of secondhand and thirdhand smoke.



While there is broad agreement among researchers and clinicians that TSE puts young children at increased risk of increased morbidity and mortality (Committee on Environmental Health et al., 2009; U.S. Department of Health and Human Services, 2014; Vanker et al., 2017; Zhou et al., 2014), little is known about the relative contribution of SHS and THS to clinical outcomes (Jacob et al., 2017). Given the pervasiveness of THS in multiunit housing (MUH) (Matt et al., 2020) the chemical composition of THS (Jacob et al., 2017), and chronic exposure risks in THS-polluted homes (Matt et al., 2011b; Matt et al., 2016), it is not surprising that initial studies show THS exposure as measured by nicotine on children's hands is associated with the presence of respiratory symptoms and respiratory-related past medical histories (Mahabee-Gittens et al., 2019). However, despite growing evidence based on laboratory studies about the toxicity and carcinogenicity of THS (Hang et al., 2020; Jacob et al., 2017; Sarker et al., 2020; Torres et al., 2018), THS-related hazards to child health have not been evaluated using markers

of contact with THS pollution and overall TSE. Thus, it is unknown how much THS exposure contributes to children's clinical illnesses and how much of the adverse effect ascribed to SHS inhalation are actually due to THS exposure. To explore these questions, we conducted a study to examine the associations of hand nicotine as a marker of children's contact with THS pollution and urinary cotinine as a biomarker of overall TSE in children of combustible cigarette smokers and their associations with child clinical characteristics including clinical symptoms and diagnoses. We hypothesized that hand nicotine and urinary cotinine levels would be strongly correlated and that both markers of TSE would be independently associated with the same respiratory (e.g., asthma) and infectious illnesses (e.g., viral infections, pneumonia). Our secondary objectives were to investigate the associations of child characteristics (i.e., sociodemographics, housing type, past medical history [PMH]), self-reported parental smoking, and child TSE patterns with hand nicotine and urinary cotinine levels. We hypothesized that younger children, children who lived in MUH, and children who live in environments where more cigarettes were smoked would have higher hand nicotine and urinary cotinine levels.

Methods

Study design

Participants were parental tobacco smokers and their children who presented to one of two Pediatric Emergency Departments (PED) or Urgent Care (UC) sites of Cincinnati Children's Hospital Medical Center (CCHMC) from April 2016 to May 2019. Child and parental dyads were enrolled in a 2-group, randomized controlled trial of a tobacco cessation intervention for caregivers who smoke called "Healthy Families" (www.clinicaltrials.gov: NCT02531594); further details are available elsewhere (Mahabee-Gittens et al., 2017). The CCHMC institutional review board approved this study; caregiver consent and child assent on children $\geq$11 years old were obtained prior to conducting study procedures.

Children were eligible for this analysis if they: were 0-17 years old, presented with potential TSE-related complaints (e.g., cough), lived with a parent who smoked cigarettes, denied use of other tobacco products (e.g., cigars, electronic cigarettes) or cannabis products, and had hand wipe samples collected during their PED/UC visit. A total of 276 participants met these eligibility criteria; only data and samples from the pre-intervention PED/UC visit, in which children were accompanied by parents who were active, daily smokers, were analyzed.

Questionnaires

During the child's PED/UC visit, parents completed electronic assessments to obtain the following sociodemographics: child: age, sex, race, ethnicity, body mass index z-score (BMIZ),

insurance type; parental highest education level; annual household income, and housing type (e.g., single-family, trailer, MUH such as townhouse, apartment building). Child weight and height were used to calculate age- and sex-specific BMIZ based on the Centers for Disease Control and Prevention 2000 growth charts (Kuczmarski et al., 2002).

Parental self-report of smoking behavior and child TSE patterns were obtained. To assess smoking behaviors, parents reported: (1) the number of cigarettes they smoked per day; (2) current daily or occasional electronic cigarette (e-cigarette) use; and (3) nicotine dependence - assessed with the Heavy Smoking Index (HSI), a validated, 2-item self-report measure (range: 0-6) derived from the Fagerstrom Test for Nicotine Dependence (Chabrol et al., 2005; Perez-Rios et al., 2009).

Parental self-report of child TSE was obtained by asking: (1) home and car smoking rules- these questions were added later in the study and *n*=111 were asked these questions; participants who reported that they never allowed smoking in the home and car were classified as having a comprehensive smoking ban (2) cumulative number of household smokers, and (3) cumulative child TSE. The latter was calculated by totaling the daily number of cigarettes smoked around the child by all smokers (e.g., mother, father, siblings, visitors, relatives) in any location (e.g., home, car).

Child Electronic Medical Record Review

Children's electronic medical records were abstracted for child clinical characteristics that served as response variables including TSE-related chief complaints (e.g., congestion/cough, difficulty breathing/wheezing, ear pain) and ICD-10 discharge diagnosis. Similar and clinically relevant diagnoses were grouped into four categories for analysis: 1) viral/other infectious illnesses: e.g., upper respiratory infection, febrile illnesses, croup, conjunctivitis, acute gastroenteritis; 2) bacterial: these were diagnoses for which antibiotics were given; e.g., otitis media, pneumonia, streptococcal pharyngitis; 3) pulmonary: e.g., asthma, bronchiolitis; 4) allergic/inflammatory or other: e.g., allergic reaction, atopic dermatitis, contact dermatitis; abdominal pain, chest pain.

Hand Wipe and Urine Collection and Processing

Hand nicotine and urinary cotinine were our primary variables of interest. Clinical research coordinators (CRC) obtained hand wipe samples by wiping the palmar and volar surfaces of all fingers of the child's dominant hand with prescreened cotton rounds (100% cotton facial wipes) wetted with 1.5 mL of 1% ascorbic acid. The methods for wipe sample preparation and nicotine analysis methods are published elsewhere (Kelley et al., 2021; Matt et al., 2021). Field blanks

were analyzed to adjust for potential contamination of the wipe samples (Mdn=1.8 ng nicotine /wipe; range 0-16.7 ng nicotine/wipe) (Quintana et al., 2013). Hand nicotine levels were reported in nanograms of nicotine per hand wipe (ng/wipe), and the limit of detection was approximately 0.19 ng nicotine/wipe. After hand wipe samples were collected, they were immediately frozen and stored at -80°C and shipped on dry ice to the analyzing laboratory (Hoh lab, San Diego State University) at which they were stored at -20°C in the dark until analysis. Hand wipe samples were analyzed by liquid chromatography- tandem mass spectrometry (LC-MS/MS). Of the 276 children with hand nicotine results, we had urinary samples available that were analyzed for cotinine in 173 (62.7%) children. Urine samples were collected, immediately frozen at -80°C and shipped on dry ice to the analyzing laboratory (Jacob lab, University of California at San Francisco) at which they were stored and frozen at −20°C until analysis for cotinine levels with LC-MS/MS using previously published methods; limit of quantitation (LOQ)=0.02 ng/ml (Jacob et al., 2011). Child characteristics differed between children with hand nicotine results only versus children with hand nicotine and urinary cotinine results based on their age, race, and PMH of a respiratory condition. Specifically, children with both hand nicotine and urinary cotinine had a higher mean (SD) age of 7.39 (0.35) years compared with children who had results for hand nicotine only (M=1.84; SD=0.30). Additionally, the group with results for both markers had a higher percentage of children who were Black and other race (p=0.02) and who had a PMH (<0.001) compared to children with results for hand nicotine only. No other between-group differences were found based on the other child sociodemographics or self-reported parental smoking and child TSE patterns.

Statistical Analyses

Hand nicotine and urinary cotinine measures underwent logarithmic transformations to address skewed distributions. We present geometric means (GeoMs), 95% confidence intervals (95%CIs), medians (Mdns), and interquartile ranges (IQRs). We initially assessed the individual associations of child characteristics, self-reported parental smoking, and self-reported child TSE patterns with hand nicotine in separate univariate regression models in all child participants. We then built similar separate univariate models with urinary cotinine as the response variable in the sub-sample of children with urinary cotinine sample results (n=173). We used Pearson correlations to assess the strength of the bivariate linear correlations between log-urinary cotinine, log-hand nicotine, child age, child BMIZ, and number of cigarettes/day smoked by parents, and cumulative child TSE (i.e., number of cigarettes smoked per day around the child by all smokers in all locations).

The hypothesized differential associations of hand nicotine and urinary cotinine for child characteristics, self-reported parental smoking, and self-reported child TSE patterns were examined in the sub-sample of children with both hand nicotine and urinary cotinine results (*n*=173) using multivariate regression analyses with both of these markers as the two response variables in one model. In addition to examining associations between child characteristics, self-reported parental smoking, and self-reported child TSE patterns with hand nicotine and urinary cotinine as the response variables, we also assessed potential interaction effects that may have influenced these markers. Prior to reaching our final model, we re-estimated models after determining model fit, possible nonlinear associations by including linear and quadratic polynomial regression terms and excluding nonsignificant explanatory variables.

Based on the differential results found in the multivariate regression model, we assessed the *n*=173 subsample to examine the associations of hand nicotine and urinary cotinine biomarker levels as the explanatory variables with clinical outcome variables as the response variables. We conducted four separate multivariable logistic regression models, including hand nicotine, urinary cotinine, and child age, race, and ethnicity as the explanatory variables, and clinical symptoms or disposition (e.g., admitted to the hospital) as the response variable. To model diagnosis group with four categories, we performed one multinomial regression model with the same explanatory variables and compared all four diagnosis groups. All statistical analyses were conducted using R version 4.0.2 (R Core Team, 2013) and Stata version 16 (StataCorp, 2019), and the Type I error was set at 0.05 (two-tailed).

Results

Sociodemographics, Parental Smoking, and Child TSE Patterns

The average (SD) child age in the overall sample (*N=276*) was 5.3 (4.9) years (Table 1). Over half of the children were male (54.0%), black (56.5%), had a household income of ≤$15,000 (64.0%), and lived in MUH or apartment buildings (55.8%). Children had a mean (SD) body mass index z-score (BMIZ) of 0.65 (1.34). Most children were non-Hispanic (97.8%) and had public insurance or were self-pay (92.8%). Parents smoked a median of 10 (IQR=6-15) cigarettes per day, and 4.7% reported current e-cigarette use (Table 2). About 19% of parental smokers had a Heavy Smoking Index (HSI) score indicating medium-to-high nicotine dependence (i.e., HSI=4-6). Over four-in-ten (42.4%) children had a home smoking ban, 36% had a car smoking ban, and only 18% had both a home and car smoking ban. Children were around a median of 2 (IQR=1-3) smokers per day who smoked a median of 4 (IQR=0-10) cigarettes/day around them in any location.

Hand Nicotine and Urinary Cotinine Levels

All children had detectable hand nicotine levels ranging from 4.0-2191.3 ng/wipe (GeoM=89.7 ng/wipe; 95%CI=[78.9;102.0], Mdn=102.6 ng/wipe, IQR=46.4-181.9). All children had detectable urinary cotinine levels ranging from 0.14-169.0 ng/ml (GeoM=10.4ng/ml; 95%CI = [8.5;12.6], Mdn=11.8ng/ml, IQR=4.4-28.1). Hand nicotine and urinary cotinine levels showed a strong positive correlation (r=0.62, p<0.001).

Bivariate Associations between Sociodemographics and Hand Nicotine (N=276) and Urinary Cotinine Levels (n=173)

Simple linear regression results indicated a quadratic relationship between age and hand nicotine with 2-4-year-olds (GeoM=133.1ng/wipe, *p*=0.001) having the highest hand nicotine levels of all age groups. (see Table 1). Black children (GeoM=13.8ng/ml, *p*<0.001) had significantly greater mean cotinine compared with White children (GeoM=5.2ng/ml). Additionally, children who had parents with an education level of ≤high school graduate/equivalent (GeoM=13.2ng/ml, *p*=0.02), had a household income level of ≤$15,000 (GeoM=13.4ng/ml, *p*<0.001), and lived in a MUH or apartment building (GeoM=12.2ng/ml, *p*=0.049) had higher urinary cotinine levels than children who had parents with an education level of ≥some college (GeoM=8.3ng/ml), had a household income level >$15,000 (GeoM=6.3ng/ml), and lived in a single-family home (GeoM=8.1ng/ml).

Associations between Parental Smoking and Child TSE Patterns and Hand Nicotine (N=276)

Children's hand nicotine showed positive linear associations with reported parental smoking (cigarettes/day) and nicotine dependence (see Table 2). While we observed a positive association between reported cumulative child TSE and hand nicotine, it was not statistically significant (*p*=0.052). Categorical patterns revealed progressively higher hand nicotine levels as the number of cigarettes/day smoked around the child by all smokers in any locations increased. Specifically, children who were exposed to 0 cigarettes/day (GeoM=73.7 ng/wipe) had the lowest hand nicotine levels followed by those exposed to 1-5 cigarettes per day (GeoM=77.6 ng/wipe *p*=0.77), 6-14 cigarettes per day (GeoM=98.1 ng/wipe, *p*=0.11), and 15-224 cigarettes per day (GeoM=158.1 ng/wipe, *p*<0.001). Children with no home ban (GeoM=113.6 ng/wipe, *p*<0.001) or no comprehensive home and car smoking ban (GeoM=99.8 ng/wipe, *p*<0.001) had higher hand nicotine levels than children with bans (GeoM=57.1 ng/wipe, GeoM=40.1 ng/wipe, respectively), independent of cumulative child TSE.

Associations between Parental Smoking and Child TSE Patterns and Urinary Cotinine (n=173)

Simple linear regression results indicated that children with parents who had medium/high nicotine dependence (GeoM=16.9ng/ml, p=0.03) had greater urinary cotinine than children with parents who had low/medium nicotine dependence (GeoM=9.4ng/ml). Children with no home smoking ban (GeoM=15.1ng/ml) had greater cotinine levels than children with a home smoking ban (GeoM=8.6ng/ml, p=0.047) while controlling for cumulative child TSE. No other significant associations were found between parental smoking and child TSE patterns and urinary cotinine.

Differential Associations between Hand Nicotine and Urinary Cotinine with Sociodemographics, Parental Smoking and Child TSE Patterns (n=160)

Multivariate regression models were used to investigate the potentially differential association of child characteristics and parental smoking and child TSE-related variables with hand nicotine and urinary cotinine. In step 1, we conducted exploratory analyses with the explanatory variables presented in Tables 1-2, examined potential interaction and nonlinear effects, and identified variables that showed no statistically significant independent contributions to either hand nicotine or urinary cotinine. In step 2, we evaluated model fit of the reduced model, examined the contribution of each explanatory variable in each model separately. In step 3, we z-standardized hand nicotine and urinary cotinine variable to compare the partial regression coefficients of each explanatory variable with respect to hand nicotine and urinary cotinine.

Table 3 shows the model estimates of the multivariate regression model. There were no significant interaction effects, and nonlinear associations were identified for age. The explanatory variables accounted for 15.6% of the variance in hand nicotine (p=0.001) and 23.9% of variance in urinary cotinine (p<0.001). In the hand nicotine model, the quadratic association with age (p=0.04) and the linear association with cumulative TSE (p<0.001) independently accounted for variance. In the urinary cotinine model, age (p=0.002), race (p=0.003), and income (p=0.008) were independently associated.

Distinct differences were noted when comparing the associated variables for hand nicotine to those of urinary cotinine (see Table 3). With respect to age, hand nicotine showed an inverted u-shape quadratic association, revealing the lower levels for children ages ≤1 year and older children ages ≥7 years compared to 1-6-year-olds. In contrast, urinary cotinine and child age showed a negative association with the highest levels for children ages ≤1 year old that declined through age 9 years and then remained at those lower levels for older children.

With respect to race, we found that hand nicotine and urinary cotinine showed significantly different associations (F(3,151)=4.97, p=0.0026). The model showed that Black children (adjusted GeoM=11.3 ng/ml, F(1,151)=18.66, p<0.001) and those of unknown race (adjusted

GeoM=25.1 ng/ml, F(1,151)= 4.9, *p*=0.03) had significantly higher cotinine levels than white children (adjusted GeoM=4.8ng/ml), independent of the other explanatory variables (i.e., child age, BMIZ, income level, cumulative TSE). In contrast, there were no differences in hand nicotine with respect to child race.

Similar to child race, there were significantly different associations between income level and hand nicotine and urinary cotinine. Specifically, children with a family income level >$15,000 (adjusted GeoM=8.0 ng/ml, F(1,151)=7.21, *p*=0.008) had lower mean cotinine than children with an income level of ≤$15,000 (adjusted GeoM=14.4 ng/ml). In contrast, there were no income-related differences with respect to hand nicotine.

Similar to the univariate findings, multivariate regression results also indicated a significant positive linear association between children's reported cumulative TSE and hand nicotine (F(1,151)=13.81, *p*=0.003). The positive association with urinary cotinine did not reach statistical significance (F(1,157)=3.64, *p*=0.058), independent of the child sociodemographics.

Differential Associations between Hand Nicotine and Urinary Cotinine with Child Clinical Outcomes

In total, 64.9% of the children presented to the UC; the remainder presented to the PED. Nearly 30% of the children had a PMH of a respiratory condition (i.e., asthma, bronchiolitis, pneumonia), and 7.6% had a PMH of prematurity. Over one-third (35.1%) presented with a chief complaint of cough/congestion, followed by 22.5% with difficulty breathing/wheezing, and 17% with ear pain. A total of 7% of children were admitted to the hospital from their PED/UC visit. The most common discharge diagnoses were viral/other infectious (48.6%), followed by bacterial (25.3%), pulmonary (21.3%), and allergic/inflammatory or other (4.8%).

We assessed potential differential associations between hand nicotine and urinary cotinine among children with both markers (*n*=173) with clinical outcome variables as the response variables (Table 4). With respect to the dominant medical complaint (i.e., cough/congestions, difficulty breathing/wheezing, ear pain) and disposition (i.e., discharged home, admitted to hospital), we examined four multivariable logistic regression models with explanatory variables as hand nicotine and urinary cotinine while controlling for child age, race, and ethnicity to predict each of the binary response variables (i.e., no, yes) and disposition. The logistic regression models showed no differences between associations of hand nicotine or urinary cotinine with chief complaints of cough/congestion, difficulty breathing/wheezing, and ear pain or disposition (all p>0.05).

We examined a multinomial regression model to assess the potential differential association of hand nicotine and urinary cotinine with four child discharge diagnosis groups while controlling

for child age, race, and ethnicity (see Table 4). Independent of child urinary cotinine levels, age, race, and ethnicity, results indicated that elevated hand nicotine levels predicted viral/other, pulmonary, and bacterial diagnoses. Specifically, with every one-unit increase in log-hand nicotine levels, children were 7.5 times more likely (95%CI=[2.1;27.2], p=0.002) to have a viral/other infectious disease diagnosis, 6.6 times more likely (95%CI=[1.8;24.4], p=0.005) to have a pulmonary diagnosis, and 5.4 times more likely (95%CI=[1.5;19.8], p=0.03) to have a bacterial diagnosis, independent of child urinary cotinine levels, age, race, and ethnicity. Urinary cotinine levels showed no associations with diagnosis independent of child hand nicotine levels and their age, race, and ethnicity.

Discussion

In this study, we present novel findings indicating that hand nicotine in children is a predictor of clinical outcomes independent of urinary cotinine. Children of smokers who have higher levels of hand nicotine are at higher risk of being diagnosed with infectious or respiratory illnesses independent of child age, race, and ethnicity and independent of urinary cotinine. In addition to its clinical implications, these findings suggest that hand nicotine is a marker of exposure to tobacco smoke pollutants that provides information additional to cotinine levels in urine. Our results expand on a previous study on a subset of this population that reported that children with symptoms of cough or congestion and a PMH of asthma or bronchiolitis have higher hand nicotine levels than those without these clinical symptoms and PMHs (Mahabee-Gittens et al., 2019). In the present study, we evaluated a larger sample of children of smokers, and we did not observe the same associations with symptoms, PMHs, and hand nicotine levels. However, the observed clinical associations suggest that THS exposure may contribute to similar illnesses in children previously thought to be associated with self-reported or cotinine-confirmed SHS exposure (Bhat et al., 2018; Strzelak et al., 2018; Vanker et al., 2017; Zhuge et al., 2020). The mechanisms responsible for these associations between hand nicotine and clinical illnesses remain unknown. However, exposure to nicotine, a constituent of THS, is known to adversely affect lung growth and function, increase airway reactivity and inflammation, and reduce mucociliary clearance in the lung; some of these effects may result in increased susceptibility to pulmonary or infectious illnesses (Chung et al., 2019; Gibbs et al., 2016; McGrath-Morrow et al., 2020). In homes where children are chronically exposed to large THS deposits on surfaces and in dust due to years of previous and/or current smoking, there are more opportunities for THS exposure via inhalation, absorption, and ingestion (Jacob et al., 2017; Matt et al., 2020). which may lead to clinical illnesses.

Although children in this study were highly exposed to tobacco smoke as evidenced by high cotinine levels, we did not observe statistically significant associations between clinical diagnoses and urinary cotinine levels. These findings are in contrast to our hypothesis and contrary to other research which has reported that self-reported or cotinine-confirmed TSE is associated with pulmonary, viral, and bacterial illnesses and other illnesses such as bronchiolitis, asthma, influenza, pneumonia, otitis media, and allergic and inflammatory illnesses (Bhat et al., 2018; Strzelak et al., 2018; Vanker et al., 2017; Zhuge et al., 2020). It is possible that we did not see clinical differences based on urinary cotinine levels because all participants in this study were children of smokers who had detectable cotinine levels, and we did not include a control group of unexposed children. Moreover, since cotinine is a biomarker of recent TSE (Benowitz et al., 2009), children in our study who were exposed days before the PED/UC visit may have had lower cotinine levels. Further, cotinine levels may vary in children based on a number of factors that do not result in variability in hand nicotine levels. These factors include: (a) metabolism: cotinine has a longer elimination half-life in infants compared to older children (Benowitz et al., 2009; Dempsey et al., 2013); b) physiologic and size characteristics: younger children have higher minute ventilation rates relative to their body mass and a higher surface area to volume ratio than older children which results in higher inhalation of SHS (Avila-Tang et al., 2013; Dempsey et al., 2013; Jacob et al., 2017); and c) genetic variability- Cotinine is formed from nicotine in a metabolic reaction catalyzed by cytochrome P450 (CYP) 2A6, and there is genetic variation by race/ethnicity and sex in the frequency of *CYP2A6* variant alleles (Benowitz et al., 2006; Dempsey et al., 2013; Zhu et al., 2013). Thus, it is possible that children who had *CYP2A6* alleles that had reduced metabolic activity may have had higher cotinine levels than those with *CYP2A6* alleles with normal metabolic activity.

In contrast, nicotine levels found on children's hands are not the result of metabolic or biological processes. Thus, we posit that hand nicotine levels may correspond more closely with children's behavior and interactions with their environment than cotinine levels. Our findings suggest that hand nicotine may provide a more direct measure of exposure to nicotine in children's environments, while cotinine may provide insights into clinical outcomes primarily associated with recent inhalation of secondhand smoke. This may partially explain why hand nicotine, as a marker of persistent THS pollution in a child's environment and thus a source of chronic exposure, was associated with the clinical illness patterns in this study, whereas urinary cotinine was not. Future studies should further investigate this supposition and assess specific markers of THS exposure and SHS exposure and clinical illness patterns in children of smokers compared to children of nonsmokers to test if these observed differential associations can be replicated. Further, although we did observe significant associations between hand nicotine

levels and certain clinical illnesses, it is important to note that of the odds ratios were relatively wide (i.e., lower and upper boundaries differed by a factor of 13) indicating the need for larger samples sizes and better overall model fit to obtain more precise estimates. Future studies should evaluate these associations in children within the same age group who have similar clinical diagnoses and illness severity classifications (e.g., children with moderate asthma exacerbation) compared to children with no significant past medical history who are clinically well.

Hand nicotine and urinary cotinine shared approximately 38% of the variance, indicating that both are markers of children's exposure to tobacco smoke and that hand nicotine may contribute to children's overall TSE (Mahabee-Gittens et al., 2018). This correlation may be partly spurious in that SHS in the air is inhaled by children and also directly partitions from the air to the skin. Findings from studies of nonsmokers without secondhand smoke exposure, however, suggest that nicotine in household dust and on nonsmokers' hands creates a separate exposure pathway for TSE independent of inhalation (Kelley et al., 2021; Matt et al., 2011b; Matt et al., 2016). These findings add further evidence that both THS exposure and SHS exposure should be evaluated when assessing the effects of overall TSE on clinical morbidity in children. Further, similar to past work (Mahabee-Gittens et al., 2019; Mahabee-Gittens et al., 2018), our findings show that children who were 2-4 years old, around more cigarettes smoked, and who did not live in homes with smoking bans had the highest levels of hand nicotine. Additionally, we report higher hand nicotine levels in children whose parents had higher levels of nicotine dependence most likely due to increased smoking in these parents. It is important to note that although children who had smoking bans in their homes and cars had lower hand nicotine levels than those without these bans, these children still picked up nicotine on their hands. Thus, smoking bans alone are insufficient to prevent THS exposure that accumulated before a smoking ban.

Similarly, children whose parents smoked fewer cigarettes had lower hand nicotine levels than parents who smoked >15 cigarettes per day. Overall, these results can be used to help motivate parents who smoke that taking steps to quit smoking, such as enforcing comprehensive smoking bans or smoking less, will potentially improve their child's health. Further, we did not observe associations with hand nicotine and race/ethnicity, parental education, or household income which underscores the importance of providing TSE reduction and tobacco cessation education for all parental smokers, regardless of their backgrounds. This suggests that children of different racial backgrounds show equivalent exposure-relevant behaviors and that the observed racial differences in cotinine result from other factors such as genetic differences in the metabolism of nicotine. Therefore, we caution against the attribution of

racial differences in cotinine levels of children to racial differences in smoking behavior of their parents.

Limitations of this study include the lack of a control group of children with no TSE, i.e., no SHS or THS as biochemically validated with hand nicotine and cotinine. Further studies should compare this control group with children of smokers who have both SHS and THS exposure with children of nonsmokers who have THS exposure only. Other factors that may have resulted in elevated THS exposure levels, such as the age of the home (Jacob et al., 2017; Matt et al., 2020) were not assessed. Similarly, some children may have had more distant exposure to active smoking depending on the timing of cotinine collection, which would have lowered cotinine levels. We may not have observed associations between clinical illnesses and cotinine because participants ranged in age from 0-17 years old, and they had a wide range of illnesses. Older children are more likely to spend more time away from home, which could have resulted in lower TSE levels compared to younger children. Further, we did not examine THS pollution present in settled house dust or surfaces in participants' homes; thus, it is unknown how closely children's hand nicotine levels matched nicotine levels in dust and on surfaces in their home environments. Lastly, we did not uniformly assess how much time had elapsed between child handwashing and the collection of hand wipes by study staff. Since more frequent handwashing can decrease the observed concentration of pollutants on hands (Li et al., 2021; Stapleton et al., 2014), there would likely be differences in hand nicotine concentrations if the timing of handwashing was considered. Note that we did find a robust and significant association between hand nicotine and urinary cotinine despite the likely influence of quasi-random differences in timing, duration, and effectiveness of hand washing. Thus, whatever the reduction in nicotine concentration was after hand washing, this alone was unable to eliminate the association between hand nicotine and urinary cotinine.

In this study of 276 children of smokers, we report that THS exposure is associated with infectious and respiratory illnesses that thus far have been considered to be associated with active smoking in the form of SHS exposure. The clinical associations observed in addition to the high correlation between hand nicotine levels and urinary cotinine levels, indicate that THS exposure may contribute to clinical findings in children. Since we did not observe any associations with diagnoses and cotinine, this suggests that hand nicotine and cotinine are not redundant markers of TSE but may provide differential insights into the source of nicotine exposure and exposure-relevant behaviors. That is, although cotinine and hand nicotine shared 38% of their variance, hand nicotine provides unique additional information about children's interaction with their environment that is not captured by cotinine. Finally, our findings are consistent with the hypothesis that exposure to THS presents a separate exposure pathway

from SHS and may be associated with clinical outcomes independently of children's exposure to SHS via inhalation. Thus, THS exposure may play an independent role in the development of tobacco-related pediatric illnesses. Further investigations in large trials of children of smokers and nonsmokers are warranted.

Table Legends

Table 1. Descriptive Statistics and Simple Linear Regression Results of Child Characteristics based on Child Hand Nicotine Levels and Urinary Cotinine Levels

Table 2. Descriptive Statistics and Linear Regression Results of Self-reported Parental Smoking and Child TSE Patterns based on Child Hand Nicotine Levels and Urinary Cotinine Levels

Table 3. Multivariate Regression Model Results Assessing the Association between Child Characteristics and Self-Reported TSE Patterns and Hand Nicotine and Urinary Cotinine Levels

Table 4. Multivariable Logistic and Multinomial Regression Results Assessing the Association between Hand Nicotine and Urinary Cotinine Levels and Clinical Characteristics

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Table 1.

Selected characteristics of the participants.

Characteristics	Exposed group	Unexposed group
No.	40	35
	Geo-mean ± SD	**Geo-mean ± SD**
Age (years)	10 ± 1.75	9.5 ± 1.95
Height (cm)	142 ± 12.5	140.5 ± 11
Weight (kg)	33 ± 7	36 ± 9
BMI (kg/m^2)	16.2 ± 1.70	18.5 ± 2
Working hours / day	10.5 ± 1.40	-
Working days / week	6.8 ± 0.41	-
Time spending outdoor per day 48 hours before sampling	14 ± 0.75	3 ± 1
Duration of using electronic devices (%)		
<1 hour	71	-
1-3 hours	20	16
3-5 hours	9	16
>5 hours	-	68
House cooling system (%)		
Air conditioner	-	64
Water cooling system	100	36
Eating fast foods (%)		
Once per week	9	56
2-3 time per week	17	28
More than 3 time	74	16
Sleep hours per day	6 ± 0.5	8 ± 1
Passive smoking (%)	Yes (43) No (57)	Yes (40) No (60)
Traffic density near the place of residence	Medium (60%) High (40%)	Medium (72%) High (28%)

Table 2.

Statistical analysis of urinary BTEX among studied groups (µg/L).

Exposure type	Before exposure					After exposure					Unexposed group			
Statistical analysis	Mean ± S.D (Min-Max)	Geo-mean	Comparison with control	Passive smokers Mean± S.D (Min-Max)	No ETS exposure Mean± S.D (Min-Max)	Mean ±S.D (Min-Max)	Geo-mean	Comparison with control	Passive smokers Mean± S.D (Min-Max)	No ETS exposure Mean± S.D (Min-Max)	Mean ± S.D (Min-Max)	Geo-mean	Passive smokers (Mean ± S.D)	No ETS exposure (Mean ± S.D)
Benzene	0.48 ± 0.13 (0.29-0.76)	0.46	p=0.53	0.52 ± 0.11 (0.38-0.71)	0.45 ± 0.13 (0.29-0.76)	1.03 ± 0.34 (0.51-1.93)	0.98	p<0.05	1.22 ± 0.35 (0.73-1.93)	0.88 ± 0.23 (0.53-1.34)	0.22 ± 0.14 (0.04-0.52)	0.17	0.24 ± 0.2 (0.04-0.5)	0.2 ± 0.12 (0.06-0.4)
Toluene	0.66 ± 0.3 (0.25-1.41)	0.6	p=0.64	0.75 ± 0.33 (0.31-1.41)	0.56 ± 0.24 (0.25-1.31)	2.16 ± 0.42 (0.51-1.93)	1.47	p<0.05	1.84 ± 0.42 (0.76-2.45)	1.35 ± 0.42 (6.6-23.5)	0.48 ± 0.18 (0.22-0.84)	0.45	0.6 ± 0.15 (0.34-0.76)	0.4 ± 0.14 (0.2-0.84)
Ethylbenzene	0.28 ± 0.11 (0.13-0.63)	0.26	p=0.71	0.36 ± 0.1 (0.25-0.63)	0.22 ± 0.07 (0.13-0.36)	0.6 ± 0.23 (0.19-0.96)	0.55	p>0.05	0.8 ± 0.12 (0.6-0.96)	0.41 ± 0.11 (1.8-6.4)	0.13 ± 0.08 (0.04-0.36)	0.11	0.18 ± 0.1 (0.06-0.36)	0.1 ± 0.05 (0.04-0.23)
o,p-Xylene	0.42 ± 0.16 (0.13-0.63)	0.4	p=0.62	0.53 ± 0.12 (031-0.77)	0.35 ± 0.16 (0.13-0.71)	1.25 ± 0.57 (0.42-2.45)	1.12	p<0.05	1.67 ± 0.48 (0.91-2.45)	0.88± 0.28 (0.42-1.47)	0.31 ± 0.1 (0.17-0.5)	0.29	0.38 ± 0.1 (0.2-0.5)	0.3 ± 0.08 (0.17-0.4)
m-Xylene	0.65 ± 0.30 (0.28-1.25)	0.6	p=0.61	0.81 ± 0.35 (0.35-1.25)	0.5 ± 0.16 (0.28-0.79)	1.38 ± 0.42 (0.61-2.14)	1.32	p<0.05	1.77 ± 0.22 (1.45-2.14)	1.03 ± 0.2 (0.61-1.31)	0.32 ± 0.12 (0.2-0.66)	0.31	0.4 ± 0.14 (0.26-0.66)	0.3 ± 0.07 (0.2-0.42)
Total BTEX*	26 ± 7.7 (14.6-45.8)	25	p=0.81	31 ± 7.4 (20.3-46)	21.8 ± 5.4 (14.6-39.8)	60.5 ± 17.9 (29.5-97)	57.8	p<0.05	75.8 ± 11.9 (49-97.14)	47.6 ± 10.2 (29-5.67)	15 ± 4 (11-24)	14.7	18.8 ± 3.3 (11.6-24)	12.8 ± 2.4 (11-19)

- BTEX unit is nmol/L.

Table 3. Urinary levels of MDA in the studied groups

Marker	Exposed group				Unexposed group	
	Before exposure		After exposure			
	Mean ± S.D (Min-Max)	Geometric mean	Mean ± S.D (Min-Max)	Geometric mean	Mean ± S.D (Min-Max)	Geometric mean
MDA (µg/g creatinine)	3.75 ± 1.4 (1.45 - 7)	3.5	12 ± 6 (2.5 - 21.7)	10.5	3.9 ± 2.1 (1 - 9)	3.3

Table 4.
Multivariate linear regression analysis of urinary BTEX (µg/l) with factors affecting exposure to BTEX in the case group [β coefficient (p-value)]

Factors	Benzene	Toluene	Ethylbenzene	o,p-xylene	m-xylene
Exposure to ETS (n/y)	**0.38 (0.03)**	**0.22 (0.02)**	**0.21 (0.04)**	**0.19 (0.02)**	**0.18 (0.02)**
BMI (kg/m^2)	-0.016 (0.6)	-0.006 (0.92)	-0.019 (0.61)	-0.013 (0.5)	-0.019 (0.29)
Traffic density in area of residence (medium regard to low)	0.042 (0.45)	0.027 (0.24)	0.013 (0.12)	0.017 (0.62)	0.014 (0.38)
Traffic density in area of residence (high regard to low)	0.11 (0.12)	0.17 (0.13)	0.091 (0.22)	0.16 (0.22)	0.17(0.33)
Creatinine (g/L)	-0.193 (0.5)	**-0.285 (0.02)**	-0.211 (0.41)	-0.118 (0.52)	-0.221 (0.81)

Table 5
Multivariate linear regression analysis of urinary MDA (µg/g creatinine) with factors affecting urinary MDA levels in the case group.

Factors	Standardized Coefficients	p-value	95.0% Confidence Interval for B	
	Beta		Lower Bound	Upper Bound
Benzene (µg/l)	**0.18**	**0.01**	**0.10**	**2.8**
BMI (kg/m^2)	0.012	0.73	-1.12	1.48
Fatigue during work (n/y)	0.038	0.55	-1.43	0.98
Eating fast foods (n/y)	0.054	0.61	-1.12	1.24
Sleep pattern	0.009	0.72	-1.18	0.081

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: