Metabolites of Exposure and Downstream Effects Found in Children Exposed to Secondhand Electronic Cigarette Vapors

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Background

Key Point: Little is known about secondhand electronic cigarette vapor (SHECV) exposure in children

- Chemicals associated with secondhand cigarette smoke (SHCS) exposure can be detected in biological specimens of bystanders
- SHCS exposure can alter endogenous chemicals in the body and negatively impact metabolic functioning and health
- Many cigarette smokers have turned to electronic cigarette use assuming less exposure for themselves and bystanders
- Minimal to no research exists establishing the chemical exposure profile of children exposed to SHECV
- Parental beliefs about SHECV exposure is unknown
- Pediatric Nurse Practitioners and other health care professionals have little evidence to guide education and interventions about SHECV exposure in children

Purpose and Specific Aims

The purpose of this research is to describe the SHECV exposure in children and parental use factors that might influence that exposure. We developed the following specific aims.

1. Compare less invasive biomatrices (saliva and exhaled breath condensate) to the gold standard, blood.
2. Detect biomarkers of exposure and downstream effects in children exposed to SHECV.
3. Describe parental use behaviors associated with child cotinine levels.
4. Identify parental beliefs about SHECV exposure in children.

Methods

Design: Two-group comparative design with follow up focus groups

Parent/Children with Exposure
- Parent uses electronic cigarette daily
- Child age 4-12 years
- Parent speaks and understands English

Note: Invited to participate in focus group

Parent/Children without Exposure
- Parent does not use electronic cigarettes or traditional cigarettes
- Child age 4-12 years
- Parent speaks and understands English

Measures: 1. Parent-completed questionnaire
2. Child blood via fingerstick (biomatrix 1 – represents the gold standard)
3. Child saliva via passive drool (biomatrix 2)
4. Child exhaled breath condensate (EBC; biomatrix 3)

Laboratory Analyses: Untargeted high-resolution metabolomics analysis on child biological samples

Note: Untargeted high-resolution metabolomics identifies all metabolic features (chemicals) in a sample. Features are then annotated (named) using validated process. Two columns are used within a mass spectrometer to analyze and yield 2 types of features; HILIC positive and C18 negative features.

Targeted analysis for cotinine levels on child saliva

Data Analyses: Descriptive statistics for sample and questionnaire results
Pair-wise correlations between biomatrices
GLM to note relationships between parent use behaviors and child cotinine
Partial least squares discriminant analysis to identify metabolic features
T-tests and Manhattan plots to identify and visualize the within-matrix differential expression of features between the 2 groups
Analysis of focus group transcripts for themes

Results

Table 1. Child Descriptive Statistics

	Cases	Controls	Difference Between Groups
Age, yrs (mean)	8.32	7.96	0.641
4-6 yrs	5	10	
7-9 yrs	9	8	
10-12 yrs	8	8	
Sex			0.683
Male	8	11	
Female	14	15	
Insurance			0.016*
Public	13	5	
Private	8	21	
Race			0.017*
Black	15	4	
White	4	17	
Asian	1	0	
More than 1 race	1	5	

Note: Insurance status and race differed between groups consistent the literature

Table 2. Parental Use Characteristics

	Frequency (%)
Times per day use e-cigarettes	
1-5	11 (50%)
5-10	4 (18.2%)
More than 10	3 (13.6%)
Continuously	4 (18.2%)
Type of e-cigarette used	
Disposable	6
Refillable cartridges	8
Refillable tank system	11
Concentration of nicotine	
1-6 mg	11 (50%)
7-12 mg	6 (27.3%)
13-18 mg	2 (9.1%)
25 mg or more	1 (4.5%)
Don't know	2 (9.1%)
Flavor of e-cigarettes used	
Menthol or mint	12
Fruit	13
Chocolate	1
Alcoholic drink	2
Candy or other sweets	8
Do you smoke cigarettes	
Every day	1 (4.5%)
Some days	12 (54.5%)
Not at all	9 (40.9%)
Number of conventional cigarettes smoked daily	
None	9 (45%)
1-5	8 (40%)
6-10	3 (15%)

Table 3. Feature Correlations Summary

HILIC Positive Features				C18 Negative Features			
Biometric Pair	Blood-Saliva	Blood-EBC	Saliva-EBC	Biometric Pair	Blood-Saliva	Blood-EBC	Saliva -EBC
Overall Corr.	0.91	0.92	0.94	Overall Corr.	0.94	0.86	0.89
Sample-Specific Corr.	0.79	0.81	0.82	Sample-Specific Corr.	0.79	0.86	0.87
Feature-Specific Raw Corr.	0.18	0.16	0.16	Feature-Specific Raw Corr.	0.17	0.16	0.15
Feature-Specific Adjusted Corr.	0.53	0.51	0.53	Feature-Specific Adjusted Corr.	0.51	0.50	0.52

Note: We compared less invasive matrices, saliva and EBC with the gold standard blood, noting moderate to high correlations

Groupings for Metabolomics Results Below:
1. Dual – parents used both electronic cigarettes and traditional cigarettes; ie, dual users
2. Ecg – parents used only electronic cigarettes
3. Case – both dual and ecg group combined

Note: Metabolites were validated with a standard from Clinical Biomarkers Lab that originally ran the untargeted metabolomics analyses

Table 4. Metabolites Indicative of Exposure that Significantly Differed from Control Group

m/z	RT	Validated Metabolite	Matrix (group; β)	Notes
101.06023	30.2	5-valerolactone	Blood (case; -0.647) Blood (ecg; -0/794) EBC (case; 0.746) EBC (dual; 1.838)	Possible flavoring agent
163.1235	36.7	(S)-nicotine	Blood (case; 2.759) Blood (dual; 2.210) Blood (ecg; 3.327) EBC (case; 1.494) EBC (dual; 1.988) EBC (ecg; 1.218)	Main metabolite of nicotine
301.2167	26.5	Retinoate	EBC (case; 0.414) EBC (dual; 0.817) EBC (ecg; 1.218)	Thickening agent in e-liquids
130.0657	30.8	Quinoline	Saliva (case; 0.596) Saliva (ecg; 0.686)	Found in electronic cigarette vapor
205.0682	59.7	Mannitol	EBC (ecg; -0.167)	Sweetening agent
667.22966	250.2	Stachyose	Saliva (case; -2627393.167) Saliva (dual; -2627393.17)	Flavoring or aroma agent
151.0237	73	Tartaric Acid	Saliva (case; 0.564)	Nicotine salt component
167.03446	22.2	Homogentisate	Saliva (case; 0.623) Saliva (dual; 0.986)	Precursor to Vitamin E
136.03988	25.8	4-Aminobenzoate	Saliva (dual; -0.451)	Possible nicotine salt component
299.20113	225.6	Retinoate	EBC (case; 0.492) EBC (ecg; 0.506)	Thickening agent
217.0484	22.2	Mannitol	EBC (dual; 0.550	Sweetening agent
89.0239	22.2	(S) – lactate	Saliva (case; -0.839) Saliva (ecg; -0.947)	Possible nicotine salt component
255.2324	27.2	Palmitoleic Acid	Saliva (case; -0.790) Saliva (dual; -0.690) Saliva (ecg; -0.863)	Fatty acid in electronic cigarettes emissions
253.21678	228	Palmitoleic Acid	Saliva (case; 0.808)	Fatty acid in electronic cigarettes emissions
133.01373	21.2	(S)-malate	EBC (dual; 0.588)	Possible nicotine salt component
147.04463	30	Trans-cinnamate	Blood (dual; 0.627)	Flavoring agent
143.10723	72.6	Caprylic acid (Octanoic acid)	EBC (dual; 0.171)	Oil in e-liquid

Table 5. Metabolites Associated with Dopamine Pathways that Significantly Differed from Control Group

m/z	RT	Validated Metabolite	Matrix (group; β)
168.10244	30.4	3-Methoxytyramine (3-MT)	Saliva (case; 0.436) Saliva (dual; 0.500) Saliva (ecg; 0.386)
180.10244	32.1	1-Methyl-6.7-Dihydroxy-1.2.3.4-Tetrahyroisoquinoline (Salsolinol)	Saliva (case; -0.758) Saliva (dual; -1.033) Saliva (ecg; -0.592)
138.09186	31.1	Tyramine	Blood (case; 0.406) Blood (dual; 0.497)

Table 6. Metabolites Associated with Oxidative Stress that Significantly Differed from Control Group

m/z	RT	Validated Metabolite	Matrix (group; β)
166.0532	88	Methionine sulfoxide (MetO)	Saliva (case; 0.410) Saliva (ecg; 0.601)
227.0663	53	Nitrotyrosine	Saliva (ecg; 1.122)

Table 7. Metabolites Associated with Inflammation that Significantly Differed from Control Group

m/z	RT	Validated Metabolite	Matrix (group; β)
328.248	27.2	Docosahecaenoic acid (DHA)	Blood (case; -1.224) Blood (ecg; -1.697)
190.05039	48.1	4-hydroxy-2-quinolinecarbosylic acid (Kynurenic acid)	Blood (case; -0.743) Blood (dual; -0.906)
137.04631	44.2	Hypoxanthine	Saliva (case; 0.730) Saliva (dual; 0.808)
731.6067	37.4	Sphingomyelin	Blood (dual; -25873.091)
154.06167	23	L-Histidine	EBC (case; 0.259) EBC (ecg; 0.294)

Table 8. Mean Cotinine Levels

Group Statistics

	CaseContr	N	Mean	Std. Deviation	Std. Error Mean
Cotinine	Control	18	.0000	.00000	.00000
	Case	20	1.7570	2.20102	.49216

Group Statistics

	CaseGroup	N	Mean	Std. Deviation	Std. Error Mean
Cotinine	E-cigarette only use	8	6325	.73653	.26040
	Dual user	12	2.5067	2.54752	.73540

Table 9. Association Between Parent Use and Child Cotinine

Factor	Sig	Adj R2
Times/Day Vaping	0.317	0.0003
Frequency of Vaping Sessions	0.987	-0.062
Nicotine Concentration	0.018*	0.234
Vaping Allowed in Home	0.054	0.175

Parental Beliefs

11 of 19 Parent users believed vaping was a minor health hazard or not a health hazard at all

12 of 22 parent users did not know if exposure to electronic cigarette vapors was harmful to children

Parent Focus Group Themes

1. Vaping as a Means to Stop Smoking

Stop Smoking Cigarettes

"Uh, I tried to quiting cigarettes. Uh, I tried the cold turkey thing and I was back on it. I tried the patches. I tried the gum. And none of it really stuck. That's when I started vaping. And, um, that was the whole reason I moved to vaping to get off of cigarettes."

Health concerns related to cigarette smoking

"Um, being that I already worked in healthcare, and then for many, many years, um, I knew that the cigarettes were bad. And, I talked to, um my doctor because at that point I was getting a little worse, like as far as my breathing and blood pressure. Um, like it was hypertensive. And I think it did have a lot to do with the cigarettes because the smoking was getting out of control. Like one pack a day was never enough. And it was-I was starting to get migraines and stuff. And, so um, you know, I researched and stuff and I also talked to healthcare provider."

2. Perceived Belief that Vaping is Better than Cigarette Smoking

Vaping is not harmful

"When I'm vaping, no, because, in my mind, I don't think that it is harmful."

Vaping is better than cigarettes

"The appeal for vaping is that at least in my mind and I say thi all the time to people that ask me I say, vapig for me is probably about 95% better than smoking cigarettes.
"For me, it seems it portrays itself as the healthier version."

Body feels better when vaping compared to smoking

"I don't have, I'm not hacking a lung every morning. I don't have the phlegm. I can run. I run miles and miles every day. I run three miles a day. I play soccer. I couldn't do any of that when I was smoking [cigarettes]."

3. Power of Addiction

Hooked on nicotine

"But its just, at this point, like, I'm-I'm hooked and I need the nicotine."

Disregard of potential harm

"I understand that putting anything foreign into my body probably isn't good for it. Um, but at that point, I didn't care. I had a fix, I wanted to fix it. It didn't really matter.

Habit addiction

"Which is still hard when you have that habit, that hand to mouth habit it is that's I think, that's what I'm struggling. Cause I'm telling myself in my head while you're like almost a zero, which means no nicotine going in so you shouldn't be that upset about it. And you know, one day I think, um, it fell on the floor an so it wouldn't work. And I mean, I-I had somewhat of a panic attack and I went to like every gas station there was immediately, you know, and I just had to find something."

Discussion/Conclusion

- Less invasive biomatrices, including saliva can be reliably used to detect exposure and downstream metabolites in children
- Various metabolites of exposure were detected in children exposed to SHECV providing evidence that the exposure is existent
- Metabolites associated with dopamine disruption, oxidative stress, and inflammation are present in children exposed to SHECV providing evidence that SHECV exposure disrupts biological processes known to lead to disease states
- Children with SHECV alone have lower mean cotinine levels compared to children with SHS and SHECV exposures indicating that children exposed to both are at higher risk
- Parental beliefs that electronic cigarette use and SHECV exposure is not harmful and parental addiction may present barriers to decreasing secondhand exposure and parental use cessation
- PNPs and other healthcare professionals must address the harmful effects of SHECV exposure in addition to SHS exposure with families

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References available upon request