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DETERMINATION OF ENVIRONMENTAL TOBACCO SMOKING IN SCHOOLCHILDREN WITH URINE COTININE MEASUREMENTS

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Summary. *Environmental tobacco smoke has been regarded as one of the most important public health issues. It has been estimated that approximately 700 millions of children in the world are exposed to environmental tobacco smoke (ETS). In this study parental smoking habits were determined. Then, the relationship between parent-reported estimates of children's exposure to ETS at home and children's urinary cotinine levels ware examined. Urinary cotinine levels were significantly higher in the exposed group than the nonexposed group. This data shows that ETS exposure was prevalent and a combination of a parent-report and biological measures is suggested as the most informative estimate of ETS exposure in children.*

Key words: *Schoolchildren, urine cotinine, ETS*

Introduction

Environmental tobacco smoke (ETS) is a material released into indoor air by smoking tobacco products, which consist of a complex mixture of thousands of compounds (gases, uncondensed vapors, tar and particle). Most of these chemical species are known as toxic or carcinogenic agents. Therefore, ETS is a group A human carcinogen as defined by the US Environmental Protection Agency.

Exposures to ETS have been associated with a variety of adverse health effects in children and non-smoking adults. Children are especially at high risk of toxicity from inhaled toxins because of differences in their pulmonary physiology and higher minute ventilatory rate (1).

According to many epidemiological studies, children's exposure to environmental tobacco smoke is a risk factor for a variety of diseases, including respiratory disorders and middle ear disease (2), but ETS can also cause injuries at the other systems and organs. Authors from Boston (3) have established association between exposure to ETS and higher prevalence of tooth caries at children (OR = 3.38; P = 0.01). Some other studies showed relationship between exposure to ETS and cognitive abilities among U.S. children and adolescents (4,5).

Measuring specific smoke constituents or their metabolites in physiological liquids can give more precise data about exposure to ETS. Although several parameters have been explored, such as carbon monoxide in exhaled air, carboxyhemoglobin in blood, thiocyanate in saliva or urine, or hydroxyproline and N-nitrosoproline in urine, urinary cotinine detection is the most frequent one (6).

Cotinine is a metabolite of alkaloid nicotine - the major component of tobacco smoke. Nicotine is metabolized in the body to cotinine and other metabolites such as 3 hydroxycotinine and nicotine N-oxide. After smoking, or exposure to nicotine from ETS, cotinine is found in blood and saliva, and, ultimately, it is expected in urine. Cotinine has a much longer half-life (~20h) than nicotine (~2h) and this is considered a more useful marker in assessing tobacco use.

Many methods have been proposed for determination of nicotine and cotinine in humane urine, plasma, hair and saliva. Cotinine measurements in biological samples have been analyzed by radio immunoassay, high performance liquid chromatography, gas chromatography usage, or nitrogen-phosphors detectors. The most frequently used method for biological samples is gas chromatography-mass spectrometry (GC-MS) (7).

The aim of this paper was to establish the relationship between parent-reported estimates of children's exposure to ETS at home and children's urinary cotinine levels.

Subjects and Methods

The study sample consisted of 1074 children aged 7 to 11 years from Nis (Serbia). Children are chosen because their lungs are even more susceptible to harmful effects from air pollutants, they aren't smokers and they aren't professionally exposed.

Anamnestic retrospective study was done first, and an original questionnaire is used. Training physicians have filled out questionnaire in direct interview with children's parents. Investigation was carried out within six months (from January to June, 2004).

The first part of the questionnaire consisted of questions about risk factors in the home environment which were associated with smoking habits. Exposure of children to ETS was specified from the following questions: "Does anyone smoke at home?", and "How many smokers live in the house?" The second part of the questionnaire was about prevalence of respiratory symptoms (nasal congestion, nasal secretion, dyspnea, wheezing, and cough), respiratory diseases (sinusitis, bronchitis, asthma, pneumonia) in children in the past 12 months.

All children were divided in a group of exposed to ETS and a group of nonexposed. In both of them, prevalence of symptoms and disease were analyzed. Interview data were analyzed using programs Epiinfo 6 and Microsoft Excel. Statistical significance of difference was established by Pearson Chi-Squared test. Odds ratio and 95% confidence interval were calculated to evaluate the presence of associations between all symptoms and diseases in children and ETS.

From the total sample of children, two groups of 15 are made: children with respiratory symptoms and respiratory diseases exposed to environmental tobacco smoke and children with respiratory symptoms and respiratory diseases nonexposed to environmental tobacco smoke. Cotinine was examined in urine of children.

Samples of morning's urine were collected from children and in the same day those were read.

«Rapid Signal COT Cassete», immunochromatographic test is used. This test is used for the rapid, qualitative detection of cotinine in urine. It is based on the principle of the highly specific immunochemical reactions between antigens and antibodies, which are used for the analysis of specific substances in urine. The cutoff of the test is 200 ng/ 1 mL of cotinine. Approximately 120 µl of urine sample is required for each test.

Test disk is removed from the foil pouch, and placed on a flat, dry surface. Two drops of specimen are squeezed into the test disk, by the sample dropper. The test result is interpreted in 5 minutes. The appereance of only one purple band within the results window indicated the result was positive, i. e. the specimen contains cotinine at a concentration above the cut-off level. The appearence of two purple bands within the result window indicated a negative test result, i. e. no cotinine above the cut-off level has been detected.

Statistical significance of difference is established by Fischer test of exact probability.

Results

In the sample of this investigation, 554 (51.60%) were boys and 520 (48.40%) were girls. The group of children ten years aged was the most numerous (Table 1).

Table 1. Characteristics of the study population

Sex	Number	(%)
Male	554	(51.60)
Female	520	(48.40)
Aged		
7	132	(12.30)
8	258	(24.00)
9	241	(22.40)
10	277	(25.80)
11	166	(15.50)

Many studies showed that great number of children have been exposed to passive smoking in households. In this investigation also, a high degree (69.65%) of exposure to this health risk factor is established (Figure 1).

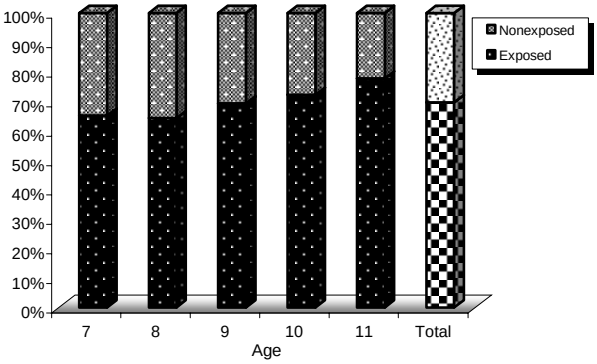

Fig. 1. Children exposure to environmental tobacco smoke

It is established that ETS was significantly associated with high prevalence of wheezing (odds ratio 1.48, and 95% confidence interval 1.09 to 2.01), as well as the prevalence of bronchitis (OR 1.66, 95% CI 1.23 to 2.23) in children (Table 2).

A total of 30 urine samples were analyzed: 6 (20%) were positive with cotinine concentrations of more than 200ng/mL. Results of cotinine detection in urine also showed that urinary cotinine levels were significantly higher in the exposed group than the nonexposed group (Table 3)

Table 2. Exposure to ETS and influence on the children health

Symptoms and diseases	Children exposed to ETS		Children nonexposed to ETS		χ^2	OR	CI	
	Yes	No	Yes	No				
Nasal congestion	589	160	238	87	3.74	1.35	0.98 -	1.84
Nasal secretion	390	359	168	157	0.01	1.02	0.78 -	1.33
Dyspnea	101	648	43	282	0.01	1.02	0.69 -	1.53
Wheezing	241	508	79	246	6.71*	1.48	1.09 -	2.01
Cough	147	602	59	266	0.32	1.10	0.78 -	1.56
Sinusitis	19	730	9	316	0.05	0.91	0.39 -	2.21
Bronchitis	277	472	85	240	11.89*	1.66	1.23 -	2.23
Asthma	23	726	8	317	0.30	1.26	0.53 -	3.08
Pneumonia	100	649	37	288	0.79	1.20	0.79 -	1.83

* $p<0.05$

Table 3. Concentrations of cotinine in urine of examined children (ng/mL)

Children exposed to ETS		Children nonexposed to ETS		
>200	<200	>200	<200	P<0.01*
6	9	0	15	

*Fischer- test

Discussion

Environmental tobacco smoke represents a serious health risk in children, whose respiratory system is still in development. The results of this investigation show strong exposure-response associations between ETS and health problems. It has been determined that children exposed to this risk factor have bronchitis and wheezing more frequent than nonexposed children.

Results of cotinine detection in urine showed that urinary cotinine levels were significantly higher in the exposed group than the nonexposed group. To our knowledge, this is the first study about the relation between exposure to ETS and urinary cotinine levels in Serbian population.

A qualitative method was used in this investigation. It is standardized and gives relevant data. The cutoff of the test was 200ng /1mL of cotinine. Detection of these values in urine of six children (20%) are worrying, especially in comparison with the other results from literature data. Namely, many studies (8, 9, 10) which were done all over the world, showed significantly higher cotinine levels in biological liquids of the exposed. For example, investigation of Scherer showed that average salivary cotinine levels in children exposed to ETS were 1.95 ng/mL, while in nonexposed 0.11ng/mL ($p < 0.01$) (11). The results of cross-sectional study in USA show that children exposed to ETS had midst cotinine level of 1.66 ng/mL (12). Mostly, urinary cotinine levels in passive smokers can be 5 to 10 ng/mL, but it is also established that these values are often higher in persons with stronger exposure (13). Typical saliva cotinine levels are 300 ng/mL for regular smokers, 100 ng /mL for occasional smokers and 0 to 2 ng/mL for non-smokers exposed to ETS (14).

Generally, cotinine levels depend of degree of ETS exposure. Increasing number of cigarettes smoked at home were predictors of increased cotinine levels (12).

The study in adolescents in Tenerife established correlation between cotinine levels in saliva and degree of ETS exposure. The highest values noticed in active smokers who smoked more than ten cigarettes daily, was 341.1 ng/mL. The smokers who smoked fewer cigarettes, average value of cotinine was 142.7 ng/mL, while in passive smokers it was 4.2 ng/mL. In the same study, it is established that infections of lower respiratory tract, persistent cough as well as bronhospasm, are more frequent in adolescents with higher concentrations of cotinine (15).

Similarly to this investigation, many others also show that more precise methods for determination of cotinine in physiological liquids allow to establish a relationship between cotinine levels and prevalence of respiratory diseases and the other health's problems. The positive relationship was determined between wheezing and salivary cotinine levels in children in Virdziny (16). Sweden group of authors have got similar results. They established that wheezing and bronchitis become worse with the increasing cotinine levels in urine (17).

Ehrilich and colleagues confirmed the positive correlation between urinary cotinine levels and prevalence of asthma in children whose mothers were smokers (18).

Conclusion

ETS is a very important risk factor for the health of exposed children. Education of parents can protect children, but determination of cotinine is more important. Monitoring of this biomarker can give proof to parents that health of their children is imperiled by exposure to ETS.

Monitoring done in this investigation can be a support for paying more attention to that way of examinees exposure. The aim of some future investigations should be quantitative determination of cotinine levels, especially when it is known that large number of children are exposed to ETS.

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ODREĐIVANJE KOTININA U URINU ŠKOLSKE DECE KAO INDIKATORA IZLOŽENOSTI DUVANSKOM DIMU

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Kratak sadržaj: *Izloženost duvanskom dimu je veoma značajan zdravstveni problem. Približno 700 miliona dece u svetu je izloženo vazduhu zagađenom duvanskim dimom. Sa ciljem da se utvrdi stepen izloženosti školske dece ovom faktoru rizika po zdravlje, najpre su praćene pušačke navike roditelja ispitanika, a zatim je vršeno merenje kotinina u urinu dece. Više vrednosti kotinina su zabeležene u urinu dece koja su u svojim domovima bila izložena duvanskom dimu. Ovaj rad pokazuje da je za preciznije utvrđivanje izloženosti dece ovom faktoru rizika najbolje kombinovati anketiranje i biomonitoring.*

Ključne reči: *školska deca, kotinin, duvanski dim*