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Evaluation of Urine Cotinine Levels in Children with Chronic Cough

Kronik Öksürüklü Çocukların İdrar Kotinin Düzeyleri İle Değerlendirilmesi

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Abstract

Aim: Chronic cough is defined as a type of cough that exists more than four weeks. This study was planned to reveal the objective relation between urine cotinine level and tobacco smoke exposure in children suffering from chronic cough.

Material and Method: Between 01.01.2012 and 01.07.2012, children aged 5-18 years who applied to the Pediatric Allergy Department with chronic cough without any etiology (patient group) and healthy volunteers of the same age group (control group) were included in the study.

Results: A total of 112 children, 58 with chronic cough and 54 healthy, were included in the study. There was a statistically significant difference between urine cotinine levels of cases of those who were exposed and unexposed (based on the declaration of parents) to tobacco smoke ($p<0.05$). The highest mean value of cotinine (41.3±73.7) was detected in the cases of the chronic cough group exposed to smoke. The best "cut-off value" for discriminating between exposed and unexposed groups was 12.15 ng/ml with ROC analysis. The smoking ratio during pregnancy was higher in the group with chronic cough ($p<0.05$). FEV1/FVC ratio was lower in patients with chronic cough who are passive smokers compared with the cases in the other groups ($p<0.05$).

Conclusions: This is the first study objectively evaluating the chronic cough effect of passive smoking with urine cotinine level. Since there is a meaningful decrease in FEV1/FVC ratio, close follow-up is needed in children, especially those diagnosed with chronic cough who are exposed to tobacco smoke.

Keywords: biomarkers, children, chronic cough, environmental tobacco smoke, passive smoking, urine cotinine level

Öz

Amaç: Dört haftadan uzun süre devam eden öksürük kronik öksürük olarak tanımlanır. Bu çalışma, nedeni saptanamayan kronik öksürüklü çocuklarda idrar kotinin düzeyi ile sigara dumanı maruziyetini objektif olarak göstermek için planlandı.

Gereç ve Yöntem: 01.01.2012 – 01.07.2012 tarihleri arasında Çocuk Alerji Polikliniği'ne başvuran 5-18 yaş arası, kronik öksürüğü olup herhangi bir etiyoloji saptanamayan çocuklar (hasta grubu) ve aynı yaş grubu sağlıklı gönüllüler (kontrol grubu) çalışmaya dahil edildi. **Bulgular**: Kronik öksürüğü olan 58 ve 54 sağlıklı olmak üzere 112 çocuk çalışmaya dahil oldu. Ebeveyn beyanına göre sigara dumanına maruz kalan ve kalmayan olguların idrar kotinin düzeyleri arasında istatistiksel olarak anlamlı fark vardı ($p<0,05$). En yüksek ortalama kotinin düzeyi (41,3±73,7) kronik öksürüğü olup sigaraya maruz kalan grupta saptandı. Sigara dumanına maruz kalan ve kalmayan grupları ayırt etmek için en iyi "cut-off" değeri (kesim noktası) ROC analizi ile 12,15 ng/ml olarak bulundu. Gebelikte sigara içme oranı kronik öksürüğü olan ve sigara dumanına maruz kalan grupta daha yüksekti ($p<0,05$). Pasif içici olan kronik öksürüğü olan çocuklarda FEV1/FVC oranı diğer gruplara göre daha düşüktü ($p<0,05$).

Sonuç: Bu çalışma, pasif içiciliğin çocuklarda kronik öksürük üzerine etkisini idrar kotinin düzeyi ile objektif olarak değerlendiren ilk çalışmadır. FEV1/FVC oranında anlamlı düşme nedeniyle, tütün dumanına maruz kalan kronik öksürüklü çocukların yakın takibi gereklidir.

Anahtar Kelimeler: Biyobelirteçler, çevresel tütün dumanı, çocuk, kronik öksürük, pasif içicilik, idrar kotinin düzeyi

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INTRODUCTION

Chronic cough is a type of cough that exists every day with a duration of more than four weeks.[1,2] Passive smoking is the exposure to tobacco smoke of a nonsmoking person through close contact or by sharing a common household.[2] In the literature, instead of passive smoking, the terms 'exposure of environmental tobacco smoke (ETS)', 'second-hand smoking', 'involuntary smoking', and 'exposure of side stream smoke' are commonly used.[1]

There is a growing amount of evidence concerning significant harms on respiratory system health about the exposure to ETS for children.[3-5] It has been reported that sharing the household with especially smoking mothers, related to the number of cigarettes smoked per day; causes recurrent respiratory system infections, wheezing, and chronic cough starting from early infancy.[6,7]

The reliability of parent declarations concerning the frequency of exposure to passive smoking is lower than objective measurements to understand the frequency of passive smoking exposure. Therefore, objective measures of biochemical markers are needed since the estimation of the prevalence of tobacco smoke exposure is probably low according to questionnaire data.[8,9] Various biomarkers are used to evaluate both active and second-hand smoking, but the most popular, specific and reliable marker is cotinine.[9-11]

Cotinine is the metabolite of nicotine, the major component of tobacco smoke.[12] Cotinine has a longer half-life than nicotine.[13] Since cotinine values in biological fluids (blood, saliva, semen, urine) are highly consistent, blood cotinine can also be accurately estimated by measuring the cotinine level in urine.[14] It is thought that the kidney concentrates cotinine, increasing the concentration in urine to levels 5-6 times higher than the concentration in plasma and saliva.[15] Various foods (such as cauliflower, eggplant, tomato, tea) contain low amounts of nicotine; however, dietary nicotine intake may be ignored in studies since it has been determined that daily intake does not cause a significant difference in cotinine levels.[14] Since urine cotinine is completely specific to cigarettes and a product of internal metabolism, the possibility of changes in cotinine levels with external environmental conditions during the collection of samples is also low.[16]

This study aimed to reveal the objective relationship between urine cotinine level and passive smoking or ETS exposure in children suffering from chronic cough.

MATERIAL AND METHOD

Selection and Collection of Study Cases

The including criteria for volunteers:

1. Children aged between 5-18 years admitted to the Pediatric Allergy and Immunology Outpatient Clinic of Dr. Sami Ulus Obstetrics, Children and Diseases Training and Research Hospital with chronic cough (duration of more than four weeks) without underlying any specific cause were included in the study (patient group). This group was also divided into two according to parents' declarations of the presence or absence of ETS (exposed/unexposed).
2. The similar age group of cases, healthy, without a history of chronic cough, and presence or absence of ETS according to parents' declarations (control group).

The exclusion criteria for volunteers:

History of prematurity, underlying chronic diseases such as chronic cardiac, renal, pulmonary diseases, chest deformities, neuromotor development retardation, malnutrition, obesity, and immune system disorder, those who lead to recurrent infections and active smokers were excluded from the study.

Study Protocol

The study design was planned as a real-life, cross-sectional trial. After getting the approval of the Ministry of Health Ankara Kecioren Education and Research Hospital's Ethics Committee on 27.06.2012 and with approval number B.10.4.İSM.4.06.68.49, the study was initiated. Written informed consent was taken from children and their parents. Detailed information was given. A standardized questionnaire form including social and demographic properties and smoking habits was applied for both children and their parents. Data was collected by the investigator by using face to face technique (by YO).

The evaluation form included the presence of cough in children, duration of cough, indoor smoking habits of parents, the part of the house parents using to smoke, the number of cigarettes per one day, smoking habit during pregnancy, education status of parents, monthly income of the family, the type of heat resources of house and number of rooms, number of individuals living in the same house and disease history of children. If indoor smoking in any area of the house is declared, this situation was considered passive smoking/ETS exposure. A family's monthly income level was evaluated by dividing it into two groups regarding the Turkish Statistical Institute Income Distribution and Living Conditions Statistics data at the time of the study: low income and normal or high income.

The parents were asked about their children's wheezing and previous respiratory system infections. Over eight upper/lower respiratory tract illnesses per year were defined as 'frequently getting ill'. Lung function testing was performed using a portable spirometry device (MIR spirolab). Children were shown how to perform the test by clamping the nostrils in standing position. Among the three measurements, the best value was taken into account. Forced vital capacity (FVC), forced expiratory volume during the first second (FEV1), FEV1/FVC ratio, forced expiratory flows between 25% and 75% of FVC (FEF 25-75%), and peak expiratory flow (PEF) were measured. Reference values were evaluated in accordance with the age of the children.[17]

Collection of Urine Samples and Laboratory Analysis

Samples of the morning's first urine were collected from the subjects included in the study. At least 5 ml of urine collected in sterile and capped urine containers with no preservatives was placed in a refrigerator at +4 °C without waiting and stored at

-20 °C within one hour at the latest. On the working day, urines were dissolved at room temperature, centrifuged at 3000 rpm for 5 minutes, and the supernatant portions were separated. The remaining sediment was gently inverted and placed in the device. Urine cotinine level was measured with Immulite 2000 Analyzer Nicotine Metabolite Device (Siemens) using solid phase competitive chemoluminescence immunoassay method. Cotinine levels were calculated in ng/ml.

Statistical Analysis

Data analysis was performed using SPSS for Windows 15.00 package program. Shapiro Wilk test was used to assess the distribution of continuous variables. In determining whether the cases were exposed to smoking or not by urinary cotinine measurements was evaluated by calculating the area under the ROC curve smoke, if there is an important area under the curve. p value <0.05 was considered statistically significant.

RESULTS

The study included a total of 112 children: 58 with chronic cough (patient group) and 54 without chronic cough (control group). Cases were divided into two groups according to exposure to passive tobacco smoke and unexposed ones according to their parents' declaration. Urine collections and analyses were performed on total 114 subjects. The urine cotinine level was measured as >500 ng/ml in two children who are active smokers indicating the exclusion criteria (parents also declared their children's active smoking status). One case with a urine cotinine level of 416 ng /ml was included in the study as a passive smoker since both the parents and himself absolutely denied active smoking.

In 59.8% of total cases, urine cotinine levels were found to be above 10 ng/ml, which was described as the threshold. The subjects that were declared unexposed to ETS smoke were found to have high urine cotinine levels (shown in **Table 1** and **Table 2**). According to parents' declarations, 32% of children without the presence of household smokers were found to have urine cotinine levels >10 ng/ml. There was a significant statistical difference between the exposed and unexposed group according to urine cotinine level (p<0.001). The best "cut-off value" for discriminating between exposed and unexposed group was 12.15 ng/ml (**Figure 1**).

Table 2. The distribution of urinary cotinine levels in children with or without chronic cough, according to the declarations of smoking status of parents

	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	p
Urinary cotinine levels (ng/ml)			
<10	11 (17.7)	34 (68)	0.442*
>10	51 (82.3)	16 (32)	

*Chi-Square Test (McNemar Test)

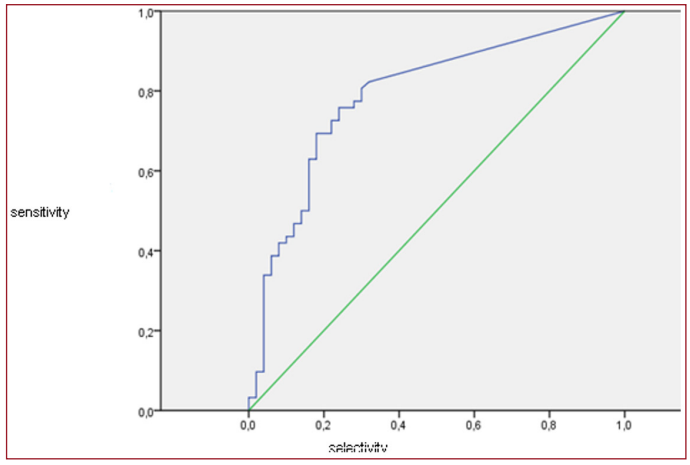

Figure 1. ROC curve of urine cotinine measurements in distinguishing children exposed and non-exposed to ETS

The demographic and social status of cases is given in **Table 3**. Considering parent's educational status, 47.5% of mothers and 32.3% of fathers were the primary school graduates. All fathers were literate and 37.4% graduated from high school. Between the groups, there was no statistically significant difference between parents' educational status (p>0.05). The cases whose mothers were primary school graduates had the most chronic cough symptom in the passive smoking group, while passive smoking rate was the least among those whose mothers were university graduates (57.1% and 7.1%, respectively). On the other hand, the most university-graduated mothers belonged to the unexposed group in the control group. However, no statistical significance was found between these two groups (p>0.05). Children with fathers of primary school graduates were the most exposed group to tobacco smoke. The mother's educational status had some influence on urine cotinine levels (p=0.042), whereas the father's educational level had none (p=0.159).

Table 1. Demographic features and urinary cotinine levels of the patients

Variables	Chronic cough group		Control group		p
	Passive smoking (+) (n=32)	Passive smoking (-) (n=26)	Passive smoking (+) (n=30)	Passive smoking (-) (n=24)	
Age (years) mean±SD	9.5±3.1	8.9±3.4	8.9±3.1	10.7±3.4	0.171*
Gender					
Male (%)	17 (53.1)	16 (61.5)	16 (53.3)	15 (62.5)	0.831**
Urinary cotinine levels(ng/ml)					
<10	6 (18.8)	15 (57.7)	5 (16.7)	19 (79.2)	<0.001***
>10	26 (81.3)	11 (42.3)	25 (83.3)	5 (20.8)	
Urinary cotinine levels (ng/ml)					
mean±SD	41.3± 73.7	13.2±5.6	23.2±13.9	17.5±25.6	<0.001*
median [min– max]	18.5[10-416]	10[10-29.6]	19.3[10-69.8]	10 [10-129]	

* Kruskal Wallis test; **Chi-Square test***;Pearson Chi-Square test

Table 3. Socio-economic characteristics of the children with and without chronic cough according to the smoking status of their parents

	Chronic cough group		Control group		p
	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	
Maternal education					
Illiterate	1 (3.6)	0 (0)	1 (3.7)	1 (4.8)	0.382*
Primary school	16 (57.1)	11 (47.8)	11 (40.7)	9 (42.9)	
Middle school	6 (21.4)	1 (4.3)	5 (18.5)	2 (9.5)	
High school	3 (10.7)	9 (39.1)	8 (29.6)	5 (23.8)	
University	2 (7.1)	2 (8.7)	2 (7.4)	4 (19.0)	
Paternal education					
Illiterate	0 (0)	0 (0)	0 (0)	0 (0)	0.343*
Primary school	7 (25.0)	7 (30.4)	9 (33.3)	9 (42.9)	
Middle school	9 (32.1)	3 (13.0)	3 (11.1)	2 (9.5)	
High school	10 (35.7)	10 (43.5)	12 (44.4)	5 (23.8)	
University	2 (7.1)	3 (13.0)	3 (11.1)	5 (23.8)	
The level of income					
Low	19 (63.3)	12 (48,0)	15 (55,6)	11 (50.0)	0.069*
Normal or high	11 (36.7)	13 (52,0)	12 (44,4)	11 (50.0)	
Home					
Poor housing	5 (15,6)	3 (11.5)	1 (3.4)	3 (13.0)	0.110*
Standard apartment	22 (68,8)	23 (88.5)	26 (89.7)	17 (74)	
Detached house	5 (15,6)	0 (0)	2 (6.9)	3 (13.0)	
Heating type					
Stove	10 (31.2)	5 (19.2)	2 (6.9)	5 (21.7)	0.149*
Natural gas	22 (68.8)	21 (80.8)	27 (93.1)	17 (74)	
Electricity	0 (0)	0 (0)	0 (0)	1 (4.3)	

*Chi-Square

There were no significant statistical differences between the groups regarding the duration, time differences and quantity of cough (p>0.05). When the household of cases of those exposed to tobacco smoke was evaluated, 47.5% of them had more than one smoker inside the houses. In the patient group, 40.6% of cases had both a smoker mother and father. This rate was 20.7% in the control group. Also in the control group, current father smokers' rate was 37.9%. There was no statistically significant difference between the exposed and unexposed groups, regarding active smokers (p>0.05). When classification was made according to urine cotinine levels, in the group of chronic cough and passive smoking, the rate of both mothers' and fathers' current smoking was the highest (46.2%). However, no statistical significant difference was found (p>0.05).

When comparison was made with the cases with chronic cough that have been exposed to ETS and other groups, the rate of smoking in pregnancy was found to be increased in the chronic cough group (31.3 % in the exposed chronic cough group, 16% in the unexposed chronic cough, 20.7% in the exposed control group, 0 % in the unexposed control) (p=0.005).

Eighty-four patients performed lung function tests. Patients with chronic cough, exposed and unexposed group according to urine cotinine level were classified and no statistically significant difference was found according to their lung function test measurements (FVC; p=0.780, FEV1; p=0.401, FEV1/FVC; p=0.709, PEF; p=0.559, FEF 25-75; p=0.709). When classification was made as pulmonary function tests of children with and without chronic cough according to the smoking status of their parents, there was a statistically significant difference for FEV1/FVC, PEF, and FEF25-75 values (p<0.05). However, for the values of FEV1 and FVC, no statistically significant difference was found between the four groups (p>0. 05) (**Table 4**).

Table 4. Pulmonary function tests of children with and without chronic cough according to the smoking status of their parents

Variables	Chronic cough group		Control group		p
	Passive smoking (+) (n=31)	Passive smoking (-) (n=25)	Passive smoking (+) (n=15)	Passive smoking (-) (n=13)	
FVC					
mean±SD	90.4±9.1	92.6±10.2	88.8±10.6	93.5±25.9	0.358*
median	89	91	84	87	
[min– max]	[74-111]	[71-115]	[74-112]	[74-176]	
FEV 1					
mean±SD	95.06±7.1	97.9±9.6	97.4±11.5	100.1±23	0.827*
median	95	95	94	96	
[min– max]	[79-108]	[80-118]	[80-117]	[83-172]	
FEV1 / FVC					
mean±SD	97.3±6.2	99.4±7.4	105.9±4.6	103.8±5.6	<0.001*
median	95.2	101	107	103	
[min– max]	[87-109]	[85-111]	[93-112]	[97-114]	
PEF					
Mean±SD	99.1±26,3	111±27.1	121.6±25.3	117.3±25.4	0.014*
median	96	105	116	116	
[min– max]	[69-192]	[73-208]	[91-169]	[81-164]	
FEF 25-75					
mean± SD	99.2±12.7	104.9±25.5	115.9±21.3	113.9±24.3	0.024*
median	97	93	109	111	
[min– max]	[75-136]	[79-189]	[86-161]	[81-177]	

Abbreviations: FVC: Forced vital capacity, FEF25-75%: forced expiratory flows between 25% and 75% of FVC; FEV1: forced expiratory volume during the first second, PEF: peak expiratory flow, *Kruskal Wallis test

DISCUSSION

In this study, we investigated the effect of passive smoking on chronic cough by evaluating 112 children (58 patient group and 54 control group). Exposure to environmental tobacco smoke (ETS) was detected in approximately two-thirds of our study group. In this study, the cut–off value to describe passive smoking was determined as 10 ng/ml. This value was under the reference of previous studies and recommended by the manufacturer.[18,19] In the chronic cough group, 55.1% of parents declared exposure to ETS, while 55.5% of parents declared exposure to ETS in the control group. According to parents' declaration, 32% of children without household smokers were found to have urine cotinine levels >10 ng/ml. These findings might indicate either a bias about parents' declarations or the fact that the children are sharing time in other smoke-free places or are subject to third-hand smoking. Previous studies showed that there could exist disparities between parents' declarations based on passive smoking rates and objective measurements. According to a survey, parents declared that tobacco smoke exposure for their children existed mainly in houses of relatives and friends, and shopping centers.[20] Although at least one smoker in the house was present, they thought they prevented their children from exposure by using different rooms for smoking. Apparently, this kind of preventive approach of parents is not proper when urine cotinine levels are measured.

For urine cotinine levels, the best sensitivity and specificity of cut–off value was 12.15 ng/ ml in our study. In the ETS-exposed group median urine cotinine levels were 18.5, while in the control group this value was 19.3 ng/ml. The highest mean cotinine level was detected in the chronic cough patient group who was exposed to ETS. Arvas et al. reported the mean cotinine value as 37.5 ng/ ml in their study.[21] Kahvecioglu et al. found median urine cotinine levels as 20 ng/ml.[22] A study investigating 7725 adult cases found that low socio-economic status increased the rate of passive smoking, and these societies have more death-end disease rates than high socio-economic status.[23] In our study, the 54.8% of cases had low income, and the majority had chronic cough and were in the group exposed to ETS. 63.3% of the group with chronic cough who were exposed to ETS was detected to be in low socio-economic status. This rate was 55.6% in the control group who had exposure to ETS. Being under more stress and having limited education of the families belonging to low socio-economic levels may increase their smoking frequency, and they may not be aware of the tobacco harm given to their children.

Exposure to ETS, while decreasing in FEV1, increases nonspecific bronchial reactivity leading to asthma development in some children. Fricher et al. reported a decrease in PEF rate values in primary school children who were exposed to tobacco smoke and found maternal smoking at a rate of 13.7%.[24] Another study evaluating maternal smoking exposure in children between ages 6-10 found a decrease in FEV1, FEV1/FVC, and FEF25-75 parameters.[25] It has also been shown that the non-asthmatic cases whose mothers had smoked during pregnancy had decreased FEV1, FEV1/FVC, and FEF 25-75 parameters.[26] Our study evaluated 84 out of the 112 patients' spirometry tests. When spirometry measures of chronic cough patients (classified according to urine cotinine level) were compared in our study, there was no statistically significant difference. However FEV1/FVC, FEF 25-75 and PEF values revealed significant differences between the groups with exposed or not. FEV1/FVC, FEF 25-75 and PEF values were decreased significantly in the children with chronic cough ($p<0.05$). FEV1/FVC values were found to be decreased in the children who had been detected as passive smokers according to urine cotinine levels or declared ETS exposure by the parents. Our results revealed that passive smoking affects lung functions negatively in children which comply with previous literature data.

CONCLUSION

This is the first study objectively evaluating the effect of passive smoking on urine cotinine levels in children with chronic cough who have no other specific underlying cause. In our study, the best sensitivity and specificity of cut–off value in urine cotinine levels was12.15 ng/ml. According to parents' declarations, some children without the presence of household smokers were found to have urine cotinine levels >10 ng/ml. These findings might indicate the importance of being subject to third-hand smoking besides wrong declarations or sharing smoke-free places. FEV1/FVC, FEF25-75 and PEF values were significantly lower in children with chronic cough exposed to ETS compared to the other groups. Therefore, children with chronic cough who are exposed to ETS should be followed up with lung function tests.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ministry of Health Ankara Kecioren Education and Research Hospital Ethics Committee (Date: 06.27.2012, Decision No: B.10.4.İSM.4.06.68.49).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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