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ARAŞTIRMA / RESEARCH

Use of urinary cotinine and cotinine/creatinine ratio as a biomarker of environmental tobacco exposure

İdrar kotinin ve kotinin/kreatinin oranının çevresel tütün dumanı maruziyetinin bir biyolojik belirteci olarak kullanımı

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Abstract

Purpose: Exposure to Environmental Tobacco Smoke (ETS) remains a worldwide public health problem. The purpose of this study was to investigate the relationship between parents' smoking habits at home and children's exposure to environmental tobacco smoke by measuring urinary cotinine levels and urine cotinine/creatinine ratios in children.

Materials and Methods: This case-control typed analytical study was conducted with 357 children in the 0-18 age group. The case group consisted of 180 children exposed to environmental cigarette smoke. As the control group, it consisted of 177 healthy children and non-smoking in their family. The levels of cotinine and creatinine in spot urinary were analyzed in both groups.

Results: The urinary cotinine level of the children was found to be statistically higher in those whose parents were smokers, female gender, fathers with a low educational level, and those with 3 or fewer rooms in the house. The urinary cotinine/creatinine ratio of the children was found to be statistically higher in those whose parents were smokers (15.91 pg/mg (1.54-147.54) vs 7.90 pg/mg (1.29-68.52)), female gender (13.19 pg/mg (1.79-115.07) vs 10.45 pg/mg (1.29-147.54)). Urinary cotinine levels in the ETS exposed group were affected 1042 times more than in the ETS unexposed group [OR:1042,462, 95% CI (139.821.839-7772.246)].

Conclusion: In the present study, urinary cotinine levels were found to be higher in children exposed to tobacco smoke than in children not exposed to tobacco smoke. In the light of these results, urinary cotinine can be used as a biomarker to evaluate exposure to ETS in children. Educating parents is essential to raising their awareness of

Öz

Amaç: Çevresel Tütün Dumanına (ÇTD) maruz kalma, dünya çapında bir halk sağlığı sorunu olmaya devam etmektedir. Bu çalışmada, çocuklarda idrar kotinin düzeyleri ve kotinin/kreatinin oranlarını ölçerek ebeveynlerin evde sigara içme alışkanlıkları ile çocukların çevresel tütün dumanına maruz kalmaları arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntem: Vaka-kontrol tipindeki analitik çalışma 0-18 yaş grubundan 357 çocuk ile yapılmıştır. Olgu grubunu çevresel sigara dumanına maruz kalan 180 çocuk oluşturdu. Kontrol grubunu herhangi bir sağlık sorunu olmayan ve çevresel sigara dumanına maruz kalmayan 177 çocuk oluşturuldu. Spot idrardaki kotinin ve kreatinin seviyeleri her iki grupta da analiz edildi.

Bulgular: Ebeveynleri sigara içenlerde, kadın cinsiyette, eğitim düzeyi düşük babalarda ve evde 3 veya daha az oda bulunan çocukların idrar kotinin düzeyi istatistiksel olarak daha yüksek bulundu. Çocukların idrar kotinin/kreatinin oranı, ebeveynleri sigara içenlerde (15.91 pg/mg (1.54-147.54) - 7.90 pg/mg (1.29-68.52)), kadın cinsiyette (13.19 pg/mg (1.79-115.07) - 10.45 pg/mg (1.29-147.54)) istatistiksel olarak daha yüksek bulundu. ÇTD'ye maruz kalan gruptaki idrar kotinin seviyeleri, ÇTD'ye maruz kalan gruptan 1042 kat daha fazla etkilenmiştir [OR:1042,462, %95 GA (139.821.839-7772.246)].

Sonuç: Çalışmamızda ÇTD'ye maruz kalan çocuklarda idrar kotinin düzeyleri, ÇTD'ye maruz kalmayan çocuklara göre daha yüksek bulundu. Bu sonuçların ışığında, çocuklarda ÇTD'ye maruziyeti değerlendirmek için idrar kotinin düzeyi noninvaziv bir belirteç olarak kullanılabilir. Ebeveynler için eğitim müdahaleleri, ÇTD'ye maruz kalma konusundaki farkındalıklarını artırmak ve özellikle ev

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exposure to ETS and teaching the right behaviors to protect children's health, especially in the home environment.
Keywords: Child, urinary cotinine, cotinine/creatinine ratio, environmental tobacco exposure.

ortamında çocukların sağlığını korumak için doğru davranışları öğretmek için gereklidir.

Anahtar kelimeler: Çocuk, idrar kotinin, kotinin/kreatinin oranı, çevresel tütün dumanı maruziyeti.

INTRODUCTION

Tobacco use is one of the major preventable causes of premature deaths and diseases in the world[1]. In fact, Among the thousands of chemicals in tobacco smoke, it contains 44 compounds classified as human carcinogens by the International Agency for Research on Cancer[2]. It is reported by the World Health Organization (WHO) that there are 1.3 billion smokers in the world and 8 million people die from causes related to smoking every year. Of these deaths, 7 million are current smokers, and 1.2 million are non-smokers who have been exposed to environmental tobacco smoke[3].

Exposure to Environmental Tobacco Smoke (ETS) remains a worldwide public health problem and is also known as 'passive smoking', 'second-hand tobacco smoke', or 'involuntary smoking'[2,4,5]. ETS is a condition caused in part by the burning end of the cigarette and the resulting smoke inhaled by non-smokers. Exposure to both active and passive smoking, the effects of which are more severe in children and newborns, can cause cardiovascular and respiratory diseases[6]. Environmental tobacco smoke is present almost everywhere smoking: at home, in the workplace, in restaurants, public buildings, hospitals, public transport, and educational institutions. Sources of exposure to smoking vary from person to person. For example, while the home environment can be a serious source of exposure for children, other sources that can contribute to exposure are schools and public transport[7,8].

When the literature is reviewed, many studies have reported that exposure to cigarette smoke has adverse effects on the health and development of fetuses and children[9]. Children are the most susceptible population to the harmful health effects of exposure to ETS[10,11]. ETS exposure risk, in many countries, including Turkey, has been managed partly by bringing public areas prohibitions on smoking. However, such a restriction does not protect the health of non-smokers if they live with one or more smokers in areas such as homes. This threat raises particular concern for the health of children due to the extraordinary vulnerability inherent in pediatric age and the longer time they spend at home with their families[12,13].

The population of Turkey is 83 million and the child population constituted 27.2% of Turkey's population[14]. Global Adult Tobacco Survey Turkey 2016 survey shows that 31.6% of adults over the age of 15 smoke tobacco. Currently, the frequency of tobacco use is higher in men (44.1%) compared to women (19.2%)[15].

Nicotine is an addictive chemical in tobacco. While nicotine itself is not a carcinogen, the quantity of nicotine intake can provide an indication of smoking behaviors[16]. Cotinine is a major metabolite of nicotine, and its urinary level is one of the most commonly measured biomarkers of tobacco smoke exposure[6,17-20].

In this study, we planned to examine the relationship between parents' smoking habits at home and their children's exposure to environmental tobacco smoke by measuring urinary cotinine levels and urinary cotinine/creatinine ratios in children. We think that our study will contribute to the national literature since there is a limited number of case-control studies investigating children's exposure to environmental tobacco smoke in our country.

MATERIALS AND METHODS

Setting and population of the study

This case-control typed analytical study was conducted with 357 children in the 0-18 age group. The case group consisted of 180 children who were admitted to the Pediatric Allergy and Immunology Outpatient Clinic due to recurrent respiratory tract infections and whose families smoked. A patient file is created for all patients who apply to the Pediatric Allergy and Immunology Outpatient Clinic and they are followed up on this file. Relevant patient files were prepared in the same order, and sections were organized including anamnesis, physical examination, preliminary diagnosis, test results, and main diagnosis – additional diagnoses of all patients. Patient files are filled in the same way for each patient in the company

of a specialist, and the diagnosis is made in the presence of a specialist. This group was evaluated as the group exposed to environmental cigarette smoke. In the control group, 177 healthy children without any health problems, no respiratory tract infections, and no smoking in their families were included. This group was selected from children who applied for periodic health examination or vaccination and were evaluated as the group not exposed to environmental cigarette smoke. The groups were kept similar to each other in terms of age and gender. The levels of cotinine and creatinine in spot urinary were analyzed from the ones included in the study in both groups.

While determining the sample of the study, the number of subjects to be included in the study was calculated using the formula n=t2. p.q/d2 since the number of individuals in the universe was not known. In line with this calculation, it was planned to include at least 328 children under the age of 18 in our study. In both groups, those with growth retardation, chronic lung disease (cystic fibrosis, etc.), history of allergy, malignancy, immunodeficiency, and gastrointestinal system diseases (reflux, etc.) were excluded from the study. In addition, children with smoking habits were excluded from the study.

A total of 197 individuals were reached in the case group. Of the individuals reached, 11 people were excluded because of the exclusion criteria, and 6 people were excluded because they refused to participate in the study. The study continued with 180 participants in the case group. In the control group, 195 individuals were reached and 18 individuals were excluded because they did not accept the study. The study continued with 177 individuals as the control group.

Before the study was started, ethical approval for the study was obtained from the Medical Faculty Ethical Committee (number: 2014/86). Participants were first verbally informed about the study, and written and verbal consent was obtained from all participants and legal heirs who accepted the study by the principles of the Declaration of Helsinki.

Data collection

A sociodemographic questionnaire created by the literature was completed by the researcher using a face-to-face interview technique with the participants to collect the necessary data for the research. Parents' educational status, occupation, average income, family type, number of children, number of rooms in the house, heating type, number of people in the house, and whether the parents smoke (smokers on the balcony were included in smoking at home) were recorded. The age and gender of the children, whether they had a congenital disease, a recognized disability, growth retardation[21], and an allergic disease were recorded.

Measurement of urinary cotinine and urinary creatinine level

After the first examination in the morning of the children participating in the study, spot urine samples were taken from the first urine of the morning and urine cotinine and urine creatinine (pg/ml) levels were measured.

The urine samples were centrifuged in a Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) brand refrigerated centrifuge at 2000 rpm for 10 minutes and their supernatants were separated. Urine samples were stored in New Brunswick U570 (New Brunswick Scientific, New Jersey, USA) refrigerator at -80 ° C until samples were studied. In the urinary samples; human cotinine (YH Biological Technology Company, Shanghai, China) was studied by ELISA (Enzyme-linked immunosorbent assay) method, and urine creatinine levels were measured by Jaffe alkaline picrate method. Urinary cotinine and urinary creatinine level were calculated according to absorbance concentration calibration by using Biotek ELX 50 microplate washer (BioTek Instruments, Vermont, ABD) and Bio-rad Microplate absorbance reader xMark (Bio-rad Laboratories, California, USA).

Urinary cotinine/creatinine ratio

Cotinine is the main metabolite of post-tobacco nicotine. The average half-life of cotinine is 19 hours[22]. The excretion of cotinine in urine may vary depending on the amount of urinary creatinine. Therefore, urinary cotinine levels were divided by urinary creatinine levels to calculate the "urinary cotinine/creatinine ratio (pg/mg) (UCCR)".

Smoking status of parents

Smokers were asked about the number of cigarettes they smoked per day and how many years they had smoked. The amount of package/year (PY) was calculated by multiplying the number of cigarette packs smoked in a day (calculated as 20 cigarettes in a pack) and the number of years he had smoked.

Those up to 19 PY were classified as "light smokers", 20-39 PY as "moderate smokers" and ≥ 40 PY as "heavy smokers".

Statistical analysis

SPSS for Windows 22.0 software (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. Descriptive statistics for continuous variables were given in terms of average and standard deviation, and descriptive statistics for categorical data were given in terms of frequency and percentage. A Chi-square test was used to compare the categorical data. The Kolmogorov–Smirnov test was used to compare quantitative data to a normal distribution. Since urine cotinine levels and urine cotinine/creatinine ratios did not show normal distribution; for statistical analysis of quantitative data, the Mann Whitney U test was used in paired groups (parents' smoking status, gender of children, etc.) and the Kruskal Wallis test was used in triple groups (occupation of the fathers). The parameters affecting urine cotinine levels and urinary cotinine/creatinine ratios were investigated using Spearman correlation tests. The independent effects of different predictors (urine cotinine/creatinine ratio and fathers' package/year quantity) on urinary cotinine levels were evaluated using a linear regression model. The model fit was assessed using appropriate residual and goodness-of-fit statistics. Correlation coefficients (r) of 0.000-0.249 were evaluated as weak relationships, 0.250-0.499 as moderate, 0.500-0.749 as strong and 0.750-1.000 as very strong. The results were evaluated with 95% confidence intervals and a significance level of $p<0.05$.

RESULTS

357 children that consisted of 51.0% (n=182) female and 49.0% (n=175) male, with a mean age of 7.61±3.94 years participated in this study. One hundred and eighty children exposed to environmental cigarette smoke and 177 children not exposed to environmental cigarette smoke were included in the study.

The education levels of the mothers were divided into two groups 49% (n=175) in primary school and below, 51% (n=182) in secondary school or higher education. There was no statistically significant difference between mothers' education levels and exposure to environmental cigarette smoke (p=0.709).

The fathers were divided into two groups 54.1% (n=193) of those with secondary education and below education level, and 45.9% (n=164) of those with high school and higher education. When the education level of fathers and exposure to environmental cigarette smoke were compared, a statistically significant difference was found between education level and smoking at home (p=0.004).

Exposure to environmental cigarette smoke was higher in those with 3 rooms or less in their house than in the group with 4 rooms or more (p=0.030). A comparison of demographic characteristics and exposure to tobacco smoke was shown in Table 1. There was no statistically significant relationship between children's gender, age, maternal education, father's occupation, number of siblings, and exposure to environmental cigarette smoke (p>0.05) (Table 1).

The urinary cotinine level of the children was found to be statistically higher in those whose parents were smokers (p<0.001), female gender (p=0.017), fathers with a low educational level (p<0.001), and those with 3 or fewer rooms in the house (p=0.024). The urinary cotinine/creatinine ratio of the children was found to be statistically higher in those whose parents were smokers (p<0.001), the female gender (p=0.050). Maternal education and number of siblings did not statistically affect urine cotinine, cotinine/creatinine ratio (p>0.05) (Table2).

A significant difference was found between the education level of the fathers and the urinary cotinine levels of the children (p<0.001). As the education level of the fathers increased, the urinary cotinine amount of the children and their exposure to cigarettes decreased. However, no statistically significant difference was found between the education level of the fathers and the urinary cotinine/creatinine ratios (p=0.083).

A moderate positive correlation was found between urinary cotinine levels and urinary cotinine/creatinine ratio (r=0.485, p<0.001). A weak positive correlation was found between urinary cotinine levels and the number of siblings (r= -0.104, p=0.050). There was a weak negative correlation between urinary cotinine/creatinine level and age (r= -0.166, p=0.002) (Table 3).

Table 1. Comparison of demographic characteristics and exposure to tobacco smoke.

Parameters	Exposure to tobacco smoke							
	ETS-exposed		ETS-unexposed		Total			
	n	%	n	%	n	%	x^2	p*
Gender of children								
Male	101	55.5	81	45.5	182	100	3.824	0.051
Female	79	45.1	96	54.9	175	100		
Age groups								
1-4 years	48	52.2	44	47.8	92	100		
5-8 years	72	51.4	68	48.6	140	100	0.504	0.918
9-12 years	34	47.2	38	52.8	72	100		
13-17 years	26	49.1	27	50.9	53	100		
Mothers'education								
≤Primary school educated	90	51.4	85	48.6	175	100		
≥Middle school educated	90	49.5	92	50.5	182	100	0.140	0.709
Fathers' education								
≤Middle school educated	111	57.5	82	42.5	193	100		
≥High school educated	69	42.1	95	57.9	164	100	8.455	0.004
Occupation of the fathers								
Civil servant	45	40.9	65	59.1	110	100		
Worker	94	56.0	74	44.0	168	100	7.185	0.066
Craftsman	35	54.7	29	45.3	64	100		
Self-employed	6	40.0	9	60.0	15	100		
Number of rooms in the home								
3 rooms and less	101	56.1	79	43.9	180	100	4.704	0.030
4 rooms and more	79	44.6	98	55.4	177	100		
Number of siblings								
≤ 1 sibling	111	54.7	92	45.3	203	100	3.416	0.065
≥ 2 siblings	69	44.8	85	55.2	154	100		

* Chi-square test was used for analysis. (ETS: Exposure to tobacco smoke)

Table 2. Comparison of urinary cotinine and cotinine/creatinine ratio with some features

Parameters	Urinary cotinine (pg/ml)	Urinary cotinine/creatinine (pg/mg)
	Median (min-max)	Median (min-max)
Parents' smoking status		
Smoker	19.20 (5.72-40.98)	15.91 (1.54-147.54)
Non-smoker	10.09 (3.79-15.27)	7.90 (1.29-68.52)
p value*	<0.001	<0.001
Gender of children		
Female	14.75 (5.32-40.98)	13.19 (1.79-115.07)
Male	12.32 (3.79-30.30)	10.45 (1.29-147.54)
p value*	0.017	0.050
Mothers'education		
≤Primary school educated	13.88 (3.79-40.98)	11.48 (1.29-115.07)
≥Middle school educated	13.26 (5.72-33.30)	11.49 (1.54-147.54)
p value*	0.378	0.869
Fathers' education		
≤Middle school educated	15.65 (3.79-40.98)	13.03 (1.44-147.54)
≥High school educated	12.49 (5.14-26.56)	9.73 (1.29-91.40)
p value*	<0.001	0.083
Occupation of the fathers		
Civil servant[a]	12.63 (5.72-40.98)	9.64 (1.79-91.40)
Worker [b]	15.51 (5.32-33.30)	11.49 (1.29-147.54)
Craftsman[c]	14.28 (6.19-26.17)	14.49 (3.43-73.04)
Self-employed [d]	10.04 (3.79-22.55)	8.97 (1.44-62.55)

p value**	0.014[ab] 0.046[ac]	0.376
Number of rooms in the house		
3 rooms and less	15.33 (5.46-25.69)	12.93 (1.29-115.07)
4 rooms and more	12.82 (3.79-40.98)	10.08 (1.44-147.54)
p value*	0.024	0.394
Number of siblings		
≥1 sibling	13.93 (5.14-40.98)	10.62 (1.29-147.54)
≤ 2 sibling	12.92 (3.79-26.39)	12.30 (1.44-68.52)
p value*	0.152	0.962
How the house is heated		
Stove	14.33 (3.79-33.30)	11.83 (1.44-147.54)
Central heating	13.02 (5.32-40.98)	11.26 (1.29-91.40)
p value*	0.348	0.846

p value*= Mann-Whitney U test *p* value**= Kruskal-Wallis test

Table 3. Correlations of some parameters

Parameters		1	2	3	4	5	6
Urinary cotinine		1					
Urinary cotinine/creatinine	r	0.485**	1				
	p	0.000					
Age	r	-0.034	-0.166**	1			
	p	0.525	0.002				
Number of the living person in house	r	-0.100	-0.088	0.193**	1		
	p	0.060	0.098	0.000			
Number of the rooms	r	-0.088	-0.006	0.020	-0.006	1	
	p	0.098	0.903	0.713	0.908		
Number of the siblings	r	-0.104*	-0.804	0.219**	0.871**	-0.049	1
	p	**0.050**	0.114	**0.000**	**0.000**	0.353	

** Correlation is significant at the 0.01 level.; * Correlation is significant at the 0.05 level.

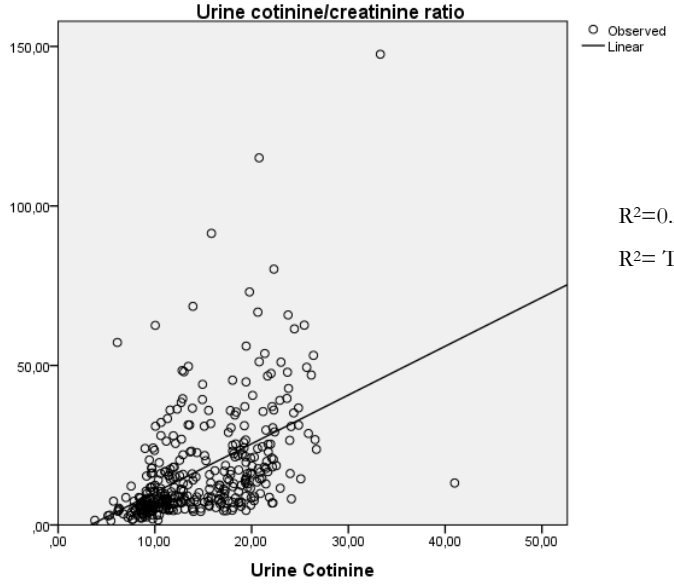
Table 4. Logistic regressions of some parameters

Parameters		OR	95% confidence interval		p
			Lower limit	Upper limit	
Urinary cotinine level	ETS-unexposed	1	139.821	7772.246	<0.001
	ETS-exposed	1042.462			
Urinary cotinine/creatinine ratio	ETS-unexposed	1	1.103	66.147	0.013
	ETS-exposed	8.186			
Fathers' education	≥High school educated	1	1.386	3.276	0.001
	≤ Middle school educated	2.131			
The number of the rooms	4 rooms and more	1	1.129	2.630	0.011
	3 rooms and less	1.723			

(ETS: Exposure to tobacco smoke, OR: Odds ratio)

When the linear regression analysis of urinary cotinine/creatinine ratio and urinary cotinine level in children was performed, 23.5% of the increase in urinary cotinine/creatinine ratio was attributed to urinary cotinine level (R2=0.235, p<0.001). (Figure 1).When linear regression analysis was performed between urinary cotinine level and father's pack/year rate; 27.0% of the highness urinary cotinine level in

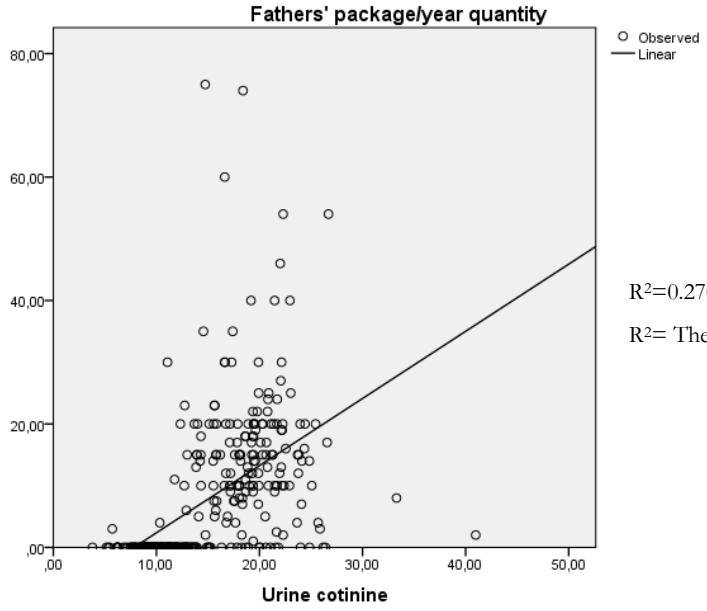
children was attributed to the father's cigarette pack/year amount (R2=0.270, p<0.001) (Figure 2). Urinary cotinine levels in the ETS exposed group was affected 1042 times more than in the ETS-exposed group [OR:1042,462, 95% CI (139.821.839-7772.246)] (p<0.001). The urinary cotinine/creatinine ratio was affected 8 times more in the group exposed to ETS than in the group not exposed to ETS [OR: 8.186, 95% CI (1.103-66.147)] (p=0.013) (Table 4).



R^2=0.235 **p<0.001**

R^2= The predictive regression coefficient

Figure 1. Linear regression analysis of the urinary cotinine/creatinine ratio and cotinine



R^2=0.270 **p<0.001**

R^2= The predictive regression coefficient

Figure 2. Linear regression analysis between urinary cotinine levels and the amount of packet/year of the father.

DISCUSSION

Exposure to ETS during childhood is a particular concern, as children exposed to toxic agents are more susceptible than adults. Public smoking bans in many countries have significantly reduced non-smokers' exposure to ETS. Despite this, indoor environments remain a very important source of exposure to environmental tobacco smoke during childhood[4]. The metabolite of nicotine taken into the body by smoking is cotinine and is excreted in the urine[9,20]. The most commonly used method to evaluate active or passive smoking is the determination of the nicotine metabolite cotinine in serum urine or saliva[23,24]. In this presented study, the effects of exposure to environmental cigarettes were investigated by comparing the urinary cotinine levels and urinary cotinine/creatinine ratios of the children who applied to the Pediatric Allergy and Immunology Clinic with recurrent respiratory tract infections and the values of the control group.

In the presented study, the median value of urinary cotinine in children was 19.20 pg/ml (5.72-40.98) in the smoking group at home, while it was 10.09 pg/ml (3.79-15.27) in the non-smoking group. Urinary cotinine levels were found to be statistically higher in children whose parents were smokers, female gender, whose father had a low educational level, and who had 3 or fewer rooms in their house. Similar to our study, Kamer et al reported that the younger age of parents, lower education level, and poor living conditions increase exposure to smoking at home[25]. In another study, likewise, ETS exposure was higher among adolescents in Kuwait, especially those with families with poor socioeconomic status and low parental education levels[26]. Unlike our study, in the study of Campo et al., urinary cotinine values were found to be higher in those with a university or higher education than in those with a high school or lower education[27].

In a study performed on 609 children who applied to an emergency, Reese et al. found that CCR levels increased in children with bronchiolitis compared to children who did not have respiratory symptoms[28]. In our study, the urinary CCR ratio was found significantly high in children who were exposed to environmental tobacco smoke at home and had recurrent respiratory tract infections when compared to the control group.

In a study by Tung KY et al. subjects exposed to ETS at home had higher urinary cotinine and UCCR than subjects who had no ETS at home[29].

In the study by Irvine et al., urinary cotinine levels of children; the age of the child, the number of smoking parents, the frequency of smoking in the same room with the child, contact with other smokers, and the crowd in the house were found to be strongly related[30].

In this study, there was a high positive correlation between the father's pack/year amount and urinary cotinine levels, and there was a weak positive correlation between the number of cigarettes smoked by the father and urinary cotinine/creatinine ratios. In the linear regression analysis between the father's pack/year amount and the child's urine cotinine level, 27.0% of the height in the urine cotinine level measured in children was attributed to the father's smoking pack/year amount. Similar to our study, Protano reported that the magnitude of ETS exposure in children was associated with parents' smoking habits and smoking precautions at home. They found that the urine cotinine levels of children living in the same house with their smoking parents increased in direct proportion to the daily smoking intensity of the parents[4]. In Wang's study, the smoking behaviors of smoking fathers and the mean duration of exposure to ETS were positively correlated with urinary cotinine concentrations of children exposed to ETS at home[16]. Although our study did not evaluate the duration of exposure to ETS at home, it was observed that the urinary cotinine levels of children exposed to ETS at home were much higher than those not exposed to ETS at home. On the other hand, the increase in urine cotinine levels with the pack/year of smoking of the father who smoked shows parallelism with this study. This significant association shows that heavy smokers not only risk adverse effects on their health, but also on the health of their children living in the same household.

The number of people and rooms at home and the bulk of the rooms are important risk factors in ETS. In a study performed by Arvas et al., no difference was found between the number and the bulk of the rooms and urinary cotinine level and urinary cotinine/creatinine ratio in the case group whose parents smoked and in the control group whose parents did not smoke[31]. Unlike this study, in our study, urinary cotinine levels were found to be higher

in children living in houses with 3 rooms or less than those living in houses with 4 or more rooms. Especially the small children being with their parents more often, the small number of rooms and the high amount of nicotine in the breathed air may cause children to be more affected by passive smoking.

Tovar et al. reported that 33.96% of children aged 1 to 60 months who attended the emergency department with acute respiratory tract infection showed a positive result for the amount of urinary cotinine that was considered positive for exposure to secondhand smoke[9].

According to the study by Susanto et al., it was shown that the urinary cotinine level in the group exposed to ETS was higher than in the group not exposed (median: ETS-exposured: 30.1 ng/ml, ETS-unexposured: 8.45 ng/ml). There was a correlation between urinary cotinine levels and the number of cigarettes smoked at home in children exposed to ETS[11]. Similar to these studies, in our study, urinary cotinine levels were high in children with ETS exposure, and the number of cigarettes smoked and urinary cotinine levels were positively correlated.

In the study of Hovanec et al. on elderly individuals, 78% of men and 80% of women reported that they did not smoke. Self-reported smoking status was high in this elderly German cohort and was not dependent on socioeconomic status[19]. In their study, Kim and colleagues examined changes in urinary cotinine concentrations in non-smoking adults after the implementation of partial smoke-free airspace regulations. Overall, urinary cotinine concentrations of Korean non-smoking adults decreased from 2012 to 2014[18]. These studies show the high level of ETS exposure and urinary cotinine level outside the home and although it has been shown that exposure can be prevented to some extent with smoke-free airspace, prevention of exposure indoors is of great importance and should be achieved through education.

This study has some limitations. The threshold value for urine cotinine to be used in international studies for children has not yet been determined. Each laboratory evaluates the results according to its method. The results of this study were based on parents' self-reported data. In this self-report, a person may report as a smoker even though they are not currently a smoker, or a "social smoker" may report that they do not smoke. There is a limited number of case-control studies in Turkey investigating exposure to ETS in children. Therefore, we think that the presented study will contribute to the national literature.

In conclusion, urinary cotinine is an easy and non-invasive biomarker that can be used to more precisely estimate the level of tobacco smoke exposure, especially in non-smokers. We think that the urine cotinine method can be used as a marker in the prediction of lung cancer risk for current smokers. In addition, urinary cotinine levels were found to be higher in children exposed to cigarette smoke than in children not exposed to indoor cigarette smoke in the presented study. In the light of these results, urinary cotinine level can be used as a noninvasive marker to evaluate exposure to ETS in children. Educational interventions for parents are essential to raise their awareness of exposure to ETS and to teach the right behaviors to protect children's health, especially in the home environment.

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