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URINARY NICOTINE METABOLITES LEVELS IN TEENAGERS

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Abstract. The **aim** of this study was to investigate the relationship between the tobacco smoke exposure (assessed by questionnaire) and the urinary total nicotine metabolites in teenagers using an accessible and rapid method and, also, to validate is method using the questionnaire in relation with nicotine metabolites levels measurements. **Material and methods.** A sample of 296 young aged 15-20 years was randomly selected from three high schools. An adequate questionnaire for assessing their smoking status was used and urine specimens were sampled for measurement of total nicotine metabolites and creatinine levels. Adequate analytical techniques were applied for accurate comparison of the two methods for tobacco smoke exposure assessment in teenagers. **Results.** A smoker frequency of 40% among the investigated teenagers was self-reported, ranging from 38% in the subjects aged 15-17 years to 50% in those aged 19-20 years. Only 22% of them were self-reported unexposed and nonsmokers and 37.6 % - passive smokers. An increasing trend was found for the urinary cotinine levels according to the duration of exposure through smoking only for those teenagers self-reported to smoke daily 5-10 cigarettes. An increasing trend was established for cotinine levels according with the smoking intensity of the subjects, with statistical significant differences between different smoker categories. The urinary thiocyanate levels did not correlate with the smoking intensity. The validation of self-reported smoker status (assessed by questionnaire) in comparison with the urinary cotinine levels has indicated a sensitivity of 0.92 and a specificity of 0.49. **Conclusions.** The findings of our study have highlighted a linear positive correlation between the urinary cotinine levels and the number of cigarettes smoked daily and, partially, with the duration of smoking exposure of teenagers. The urinary cotinine level, as exposure bioindicator, was proved to be a useful tool for measurement of organism impregnation, as smoking consequence. The techniques of validation by the help of questionnaire have indicated that is a sensitive but less specific method for exposure assessment in the comparison with the major urinary nicotine metabolite measurement.
Key words: tobacco smoking, teenagers, urinary nicotine metabolites, validation

Rezumat. Obiectivul acestui studiu a fost investigarea relaţiei dintre expunerea la fumul de ţigară (estimată prin chestionar) şi nivelul metaboliţilor urinari ai nicotinei la adolescenţi şi, de asemenea, validarea metodei prin chestionar, în raport cu nivelul bioindicatorilor de expunere. **Material şi metodă.** Un lot de 296 de adolescenţi cu vârsta cuprinsă între 15 şi 20 ani a fost selectat intâmplător din trei licee. A fost utilizat un chestionar adecvat pentru investigarea statusului de fumător/nefumător. S-au aplicat tehnici analitice corespunzătoare pentru compararea celor două metode de evaluare a expunerii adolescenţilor prin fumat. **Rezultate.** Frecvenţa fumătorilor la lotul investigat a fost de 40%, crescând de la 38% la vârstele de 15-17 ani până la 50% la 19-20 de ani. Doar 22% dintre adolescenţii investigaţi s-au declarat nefumători neexpuşi iar 37% ditre ei s-au declarat fumători pasivi. S-a constatat o tendinţă de

creştere a nivelului cotininei urinare, cu diferenţe statistic semnificative între diferite categorii de fumători. Nivelul tiocianaţilor urinari nu s-a corelat cu intensitatea fumatului. Validarea statutului auto-declarat de fumător (estimat prin chestionar) în raport cu nivelurile cotininei urinare au indicat o sensibilitate de 0,92 şi o specificitate de 0,49. **Concluzii.** Rezultatele studiului au evidenţiat o relaţie lineară între nivelul cotininei urinare şi intensitatea fumatului, şi, parţial, cu vechimea statutului de fumător. Măsurarea nivelurilor cotininei urinare, ca bioindicator de expunere, s-a dovedit un instrument util pentru măsurarea impregnării organismului, ca o consecinţă a fumatului. Tehnicile de validare a metodei cu ajutorul chestionarului au arătat că aceasta este o metodă sensibilă dar mai puţin specifică în evaluarea expunerii în raport cu măsurarea metabolitului principal al nicotinei în urină.

Cuvinte cheie: fumat, adolescenţi, metaboliţi urinari ai nicotinei, validare

INTRODUCTION

According to World Health Organization (WHO), tobacco smoking is one of the main causes of morbidity/mortality, especially by cardiovascular diseases and lung cancer (1). Smoking contributes to diminution of life expectance, too. For children and young people, tobacco smoking habit generates higher risk for complications of upper respiratory airways infections and an important respiratory insufficiency (2-7). On the other hand, passive smoking was classified in the list of potential carcinogenic agents by US Environmental Protection Agency (8). The survey of behavior pattern with risk in teenagers has indicated an increase of smoker prevalence with 2 % per year, over the last decade in our country.

The knowledge of smoking frequency, among other behaviors with health risk represents one of the topics investigated by our specialists in the recent years, in studies on teenager's samples from different schools in Iaşi town. The results of the studies carried out in 2000-2002 period on a sample of 837 school children aged 15-22 years, have revealed a smoking frequency of 42.6 % and a poor information about the risks associated. The most mentioned smoking start-age declared by the investigated subjects was 14-15 years for boys and 16-17 years for girls.

The most used method for smoking exposure estimation is based on questionnaire, despite the biases by: underestimation of exposure, differences in nicotine content and not taking into account the depth of tobacco smoke inhalation. This is the reason why more objective methods – accurate, relatively cheap biochemical measurement of some exposure bioindicators in biological samples are used in the recent years in order to quantify the organism impregnation as a smoking consequence (9-15).

In this context, our study aimed:

1. to investigate the relationship between tobacco smoke exposure (assessed by questionnaire) and the urinary nicotine metabolite levels in teenagers;
2. to validate the method using the questionnaire versus exposure bio-indicators levels.

MATERIAL AND METHODS

A sample of 296 young aged 15-20 years was randomly selected from three high schools from Iaşi, Bacău

and Galați towns. Most of them were 17-18 years old.
An adequate questionnaire (validated by a pilot study) was used for assessing their smoking status and urine specimens were sampled for measurement of total nicotine metabolites and creatinine levels.

Questionnaire (anonymous)
1. Gender M/F…… 2. Age (years turned)……3. Are you a smoker? Yes/No.
4. How long have you smoked?……
5. Now you smoke: regularly (daily)/ on occasions……
6. No. cigarettes/day: 0-4; 5-9; 10-19; 20 and over
7. If the case, how many hours a day do you spend in an environment polluted by cigarette smoke by other smokers (family or friends):
8. No. hours/24 hours…..
9. Months or years of exposure……..

The measurements in urine specimens were as follows:
- Total nicotine metabolites (TNM) (expressed as cotinine equivalents) using a spectrophotometric method (13).
The performance characteristics of the method we used were:
- Reproductibility (within test): 5.6% (n = 20)
- LOQ: 0.07 μmol cotinine equivalents/L
Quality Control: all the analyses were undertaken in duplicate and "blind", without reference to the self-reported smoking status of the subjects.
- Creatinine using alkaline picrate in Jaffe reaction (14-15).

RESULTS AND DISCUSSION
The study lot included more girls (n=182) than boys (n=114), most of them aged 17-18 years (table 1).
The frequency of smokers in the overall group was of 40.2 percent, increasing from 38.2-40.9% at 15, 16 and 17 years of age ant to 50.0% at 19-20 years (fig. 1).

Table 1. Age and sex-distribution of the study group

Age (years)	Boys	Girls	B + G
15	9	1	10
16	15	19	34
17	48	88	136
18	39	62	101
19	2	12	14
20	1	-	1
Total	**114**	**182**	**296**

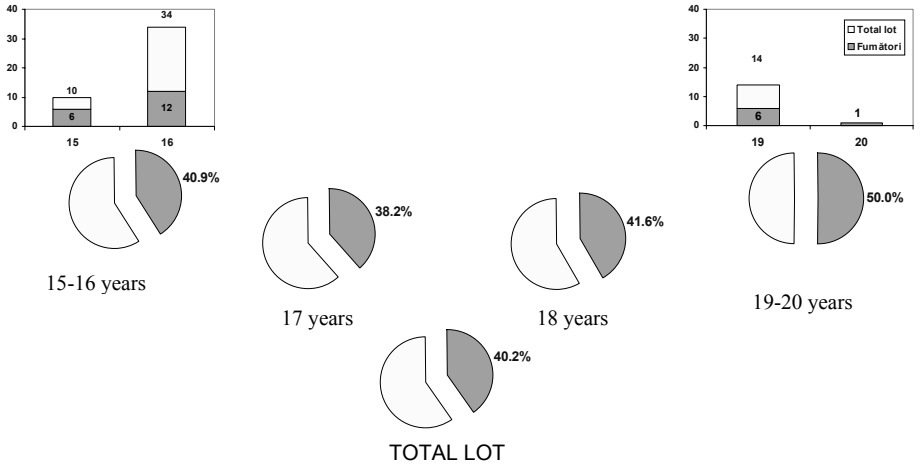

Fig. 1 Age-distribution of the number/frequency of smokers in the study lot

As to the intensity of smoking (7 categories), 52.6 percent of the boys consider themselves smokers, as compared with 32.4% of the girls; also, more boys smoke 5-9 and 10-19 cigarettes/day than girls (fig. 2).

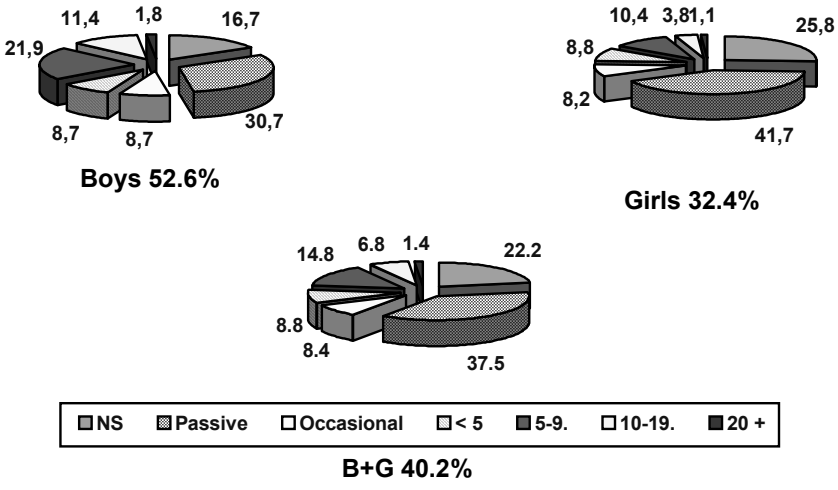

Fig. 2 Sex-distribution of teenagers according to the intensity of smoking

Only 22.2% of the subjects declare to be non-exposed non-smokers (more girls than boys), and 37.5% passive smokers (also more girls than boys) (fig. 3).

By analyzing the relationship between urinary TNM levels and the length of smoking, an increasing tendency of urinary TNM was noticed but in the subjects who declared smoking 5-9 cigarettes/day (figures 4 and 5).

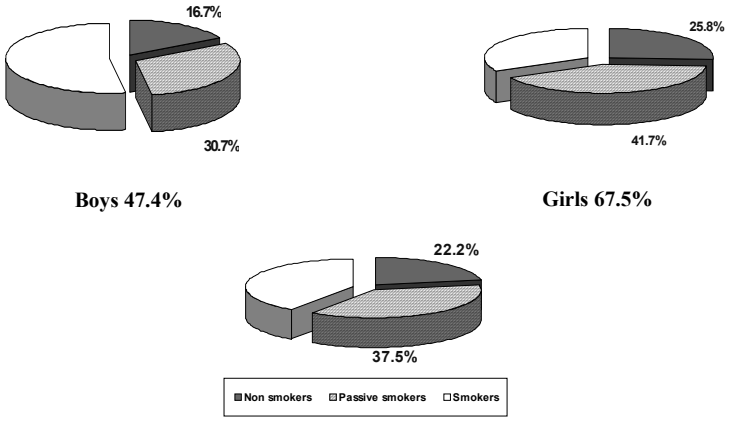

Fig. 3 Sex-distribution of the frequency of non-smokers and passive smokers in the study group

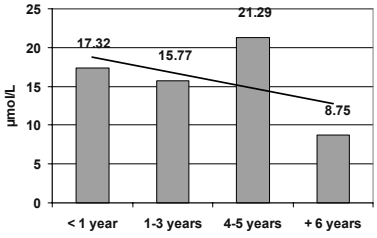

Fig. 4 Urinary TNM levels (µmol/L) according to the length of smoking (< 5 cigarettes/day)

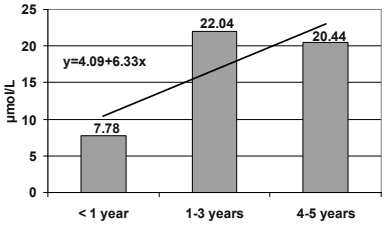

Fig. 5 Urinary TNM levels (µmol/L) according to the length of smoking (5-10 cigarettes/day)

A tendency of urinary nicotine metabolites levels to increase was found depending on the intensity of smoking (figures 6 and 7). Statistic differences between passive and occasional smokers, occasional smokers and those who smoke 0-5 cigarettes/ day, and that smoking 10-19 cigarettes/day are significant. The same is true for the levels of nicotine metabolites adjusted with urinary creatinine.

The validation of self-reported smoker status in comparison with the urinary cotinine levels has indicated a sensitivity of 0.92 and a specificity of 0.49.

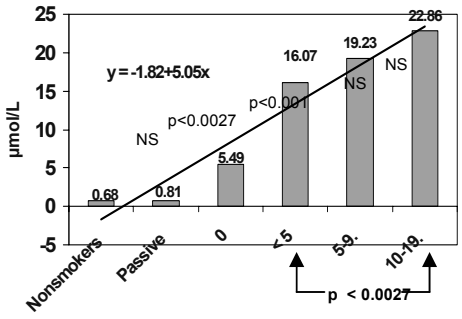

Fig. 6 Urinary TNM levels (µmol/L) in the teenagers under study according to the intensity of smoking

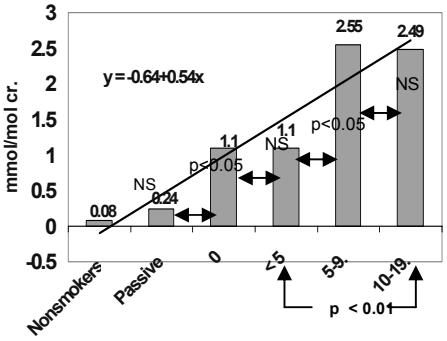

Fig. 7 Levels of urinary TNM adjusted with creatinine (mmol/mol cr.) in the study group according to the intensity of smoking

CONCLUSIONS

The findings of our study have highlighted a linear positive correlation between the urinary nicotine metabolites and the number of cigarettes smoked daily and, partially, with the duration of smoking exposure of teenagers.

The urinary nicotine metabolite level was proved to be a useful tool in order to measure the body intake, as smoking consequence, in the estimation of exposure associated with this risk.

The validation of self-reported smoker status in comparison with the urinary cotinine levels has indicated a sensitivity of 0.92 and a specificity of 0.49.

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