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Original Contribution

SMOKING AND THIOCYANATES IN SCHOOLCHILDREN

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ABSTRACT
This survey is related to evaluation the concentration of thiocyanates in urine as a marker of smoking as tested in 84 clinically healthy university students aged 18-20 years and 86 school students aged 15-18 years.
Values obtained for the concentration of thiocyanates in urine of school children in non-smokers are - 25,46 ± 6,19 µmol / l, in school students who smoke 10 cigarettes a day - 62,78 ± 14,10 µmol / l and in smokers over 10 cigarettes a day - 133.99 ± 31,65 µmol / l.
The results showed that 84.88 percent of the surveyed school children were smokers and 29.07% of them smoke more than 10 cigarettes a day.
The renovated and computerized spectrophotometric method used in this study for determining the thiocyanates in urine samples is suitable for serial analysis.
The seriousness of health and social problem of smoking is confirmed on the basis of an objective quantitative marker for distinguishing smokers from non smokers.

Key words: thiocyanate, tobacco smoking, biomarker

Tobacco smoke

Tobacco smoke contains over 4 000 chemical compounds of which over 100 are considered to be harmful for the health. That includes about 50 - 60 carcinogens, mutagens and some very irritating or toxic substances, like a small quantity of the deadly polonium-210 isotope. It is reported that a puff of cigarette smoke contains 1016 oxygen radicals. It was found that tobacco smoke can cause toxic damage to cells. Cigarette smoke and its oxygen radicals activate inflammatory cells that contribute to the activation of growth factors on matrix metalloproteinases. This causes a protease - antiproteases imbalance and destruction of tissue (1).

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Tobacco smoke enters air when the smoker does not inhale the smoke into his lungs called sidestream smoke and also enters air when a smoker exhales smoke called basic smoke. The share of sidestream smoke is 50 - 90 % and the proportion of basic smoke is 10- 50 %. Side stream smoke is particularly dangerous for health because it contains more harmful compounds than the basic smoke. When its particles are mixed with indoor air, they become smaller in size and are assigned to the so-called inhaled particles (2).

Smoking and Health

Smoking has an adverse impact on development and health of adolescents. Tobacco smoke contains many toxic substances that are harmful for both active and passive smokers.

Smoking affects the growth of the fetus of pregnant women who smokes. Newborn infants of smoker mothers are with mild retardation in neuro-psychological development and decreased cognitive abilities (3, 4).

Number of smokers is increasing among adolescents and children. The peak of early smoking is shifting from 18 years to 14-16 years of age and first experiences of smoking occur in the first decade of development of children.

Structural changes in DNA are observed among active and passive smokers in puberty. This is associated with infertility, reproductive failure and subsequently giving birth to disabled children. When exposed to passive and active smoking in childhood and adulthood these cancers are common - lung, throat, esophagus, pancreas, kidney, bladder, cardiovascular diseases, including heart attack, cerebrovascular diseases and stroke. Other problems caused by smoking are an increased risk of high blood pressure, lung diseases, sexual problems, including infertility, various allergies, worse oral health - teeth, gums, mouth ulcers, smoking can cause macular degeneration and gradual loss of vision is increased in smokers and risk of cataracts. (5-8).

Although smoking causes many health problems special attention should be given to its effects on the respiratory system - emphysema, pneumonia, chronic bronchitis, asthma. (9, 10).

The components of tobacco smoke cause non-specific inflammation of the bronchial mucosa in which along with swelling there is increased mucous secretion. Increased bronchial reactivity and bronchial obstruction are associated with a greater frequency of asthma among children who smoke or are exposed to passive smoking (2, 11–13).

Assessment of smoking

Often information about the statistical evaluation of smoking is given by surveys. Results depend on many factors and are not always reliable. Quantitative analytical criteria are used to distinguish smokers from non-smokers and reported intensity of smoking. Hypothetically ideal marker should be specific for the source of smoke, its concentration should increase in proportion to the consumption of cigarettes, also should be easily measured by standard techniques and techniques for the quantification must be operationally easy, economical and sensitive. On the other hand, measured concentration should not be influenced by tobacco brands, as well as to obtain statistically significant difference not only in smokers and non-smoker, and to take account of the intensity of smoking.

Markers of smoking are divided into two main groups: weather and biomarkers. Briefly, biomarkers are: carbon monoxide, nicotine, cotinine, nornikotin, thiocyanate, and anabazin anatabin, solanezol (Solanesol), nitrosamines, 4-aminobiphenyl, 1-hydroxypyrene, 5-nitro-gamma-tocopherol, N-(2-hydroxyethyl)-valine. (14, 15).

Indicators for distinguishing smokers from non smokers and treatment of smoking, most often include measuring the level of carbon monoxide (CO, COHb), nicotine (in plasma or serum, saliva, urine, hair, nails) cotinine (plasma, saliva, urine, hair), thiocyanates (plasma, saliva, urine) and anabazin anatabin (not used in pharmacological preparations as Anabasine anatabine or in serum, urine) (16 - 24). The aim of the present research was to evaluate the intensity of smoking and the ratio (%), between the number of smokers and non smokers among students using a method for determination of the concentration of thiocyanates in urine as criteria to distinguish smokers from non smokers.

MATERIAL AND METHODS

Selection of the studied groups

To determine the normal levels of thiocyanates in urine and to evaluate their elevated values as a result from the smoking two main groups of young people were selected. First group consisting of 84 medical students were used as clinically healthy controls (25). Medical students were divided into smokers and non smokers, based on a survey conducted on a voluntary basis for credibility and objectivity of data. Tested students are young adults who are independent. As future physicians, we believe that students have volunteered consciously to medical study, which is related to the survey. It provides information on the number of cigarettes smoked per day.

The age of the healthy controls (18 - 20 years) was close to that of the other tested group (15 - 18 years) consisting of 86 secondary school children. School children were split into three groups: smokers, smoking up to 10 cigarettes a day and smoking more than 10 cigarettes a day, based on results obtained for the content of the thiocyanate in urine samples. Students were examined with questionnaire after the completion of quantitative research.

In one and the same day in morning the urine samples were collected and studied for determination of thiocyanates as a marker for smoking.

Spectrophotrometric method for determination of thiocyanates in urine samples

Determination of thiocyanates in urine samples is a noninvasive assay and is a proper marker to distinguish smokers from non smokers. The concentration of thiocyanates in biological samples can be determined by various methods - gravimetric, titrimetric, electrochemical, kinetic, chromatographic, atomic, radiofluorometric, spectrophotometric (21, 23, 26-31). For the present survey we have used the method of Girandi and Grillo (32) with our modifications. This method is one of the commonly used spectrophotometric methods (21, 31, 33 - 35). It possesses some advantages over the others because is not necessary thiocyanates to be separated and concentrated preliminarily, and the presence of the other ions in the sample do not affect the main reaction. The low detection limit of the method allows working with less quantity of urine and reagents. Test relative standard deviation (RSD% - Relative Standard Deviation) showed good reproducibility of the method 2.04 % to 6.00 %. This method is fast and its technical performance is easy – requires adding two reagents to pre-treated samples, which makes it applicable to serial analysis.

Some authors such as Swan GE et all (36), consider that there are products like cane, almonds, nuts, beer, cauliflower, broccoli, sweet potatoes etc contain thiocyanates which can affect the marker for smoking. Other authors like Galanti LM (37), reported that the concentration of thiocyanates in biological samples is not affected by endogenous intake of thiocyanates. To avoid this short of conflict a control group of smokers were also included in the survey.

Experimental

Reagents

All solutions were prepared from analytical – reagent grade substances and double distilled water. Laboratory glassware was immersed for 12 h in hydrochloric acid: water (1:1), washed ten times with distilled water and five times with double distilled water and dried at 140°C. The glassware was kept in vacuum closed vessels before and after the taking samples.

Primary solution of potassium thiocyanate (KSCN - Merk KGaA) was prepared by mixing 0.167g of thiocynate in 100cm^3 of double distilled water. Standard solutions of 0.1, 0.3, 0.5 mg /l, were prepared from primary solution of potassium thiocyanate. Acetate buffer, pH 4.1 is prepared by adding 10.8 g sodium acetate ($CH_3COONa \bullet 3H_2O$ - Merk KGaA) in 24 ml glacial acetic acid (CH_3COOH - Fulka AG) and diluted to 1 liter with double distilled water. Reagent **K** is a clear filtrate of 1:1 mixture of 1% chloramin T trihydrate ($C_7H_7ClNaNO_2S \bullet 3H_2O$ - Merk KGaA), and 0.1% ferric chloride ($FeCl_3 \bullet$ 6H2O - Merk KGaA). The two solutions were mixed and left for 12 h. The mixture was filtered. Reagent **P** is obtained as a soluble 6 g of barbituric acid (C4H4N2O3 - Merk KGaA) and 64 g of double distilled water, then added 6 ml of concentrated hydrochloric acid (HCl - Riedel-de-Haën, d = 1.18). Shelf life of reagents **K** and **P** in the 0 - 4 ° C is about a month.

Apparatus

A Specol 11 spectrophotometer with EK – 5 temperature- controlled cells and a PHM 64 research pH were used.

Spectrophotometric determination of thiocyanates in urine

Analysis of urine samples is preceded by pre-treatment. In dry tubes with glass covers 0.25 ml urine and 1 ml acetate buffer were mixed, double distilled water was added to bring volume to 5.00 ml.

For the determination of thiocyanates 1 ml of diluted urine samples was added to 1 ml of reagent **C**. The sample was mixed well for 20 - 30 s. Then 0.4 ml reagent **R** was added and mixed again. Absorptions of the samples were measured after 15 - 20 min incubation at room temperature at 605nm. For the calibration of graph for absorption vs. above concentration of thiocyanates (0.1, 0.3, 0.5 mg / l SCN^-), 1 ml of standard solutions was mixed with reagent **P** and **K**, and absorption was measured by already above described method. Data obtained by spectrophotometric absorbance of standards and sample is submitted to the computer and processed in the program Excel. Based on values for absorption of urine samples the calibration graph is constructed by electronic means of the concentration of the thiocyanates. The calibration graph is checked before each test series.

STATISTICAL ANALYSES

Excel (Microsoft Corporation, Redmond, WA) and Statgraphics Plus (Manugistics, Rockville,MD) for Windows were applied in order to be analyzed statistically the results of the study.

All values were expressed as mean (X) ± standard deviation (SD). Student's t-test, Ratio, and one-way analysis of variance (ANOVA) was used. Correlation analysis was performed for selected data sets, and data were considered significant when p value was less than 0.05 (p).

RESULTS AND DISCUSSION:

The results of the study with medical students are presented in **Table 1**. The values of thiocyanates calculated from the studied urine samples in smokers and nonsmokers were similar to those reported by other authors (16, 30).

Table 1. *Content of thiocyanates in urine samples of medical students*

Tested / Indicators	Nonsmokers	Smoking up to 10 cigarettes per day	Smoking more than 10 cigarettes per day
Number	42	23	19
Thiocyanates (X±SD), μmol/l	23,25 ± 8,61	55,97± 27,72	147,06 ± 59,24
Statistical reliability		p< 0,001 p< 0,001	

After determining the concentration of thiocyanates in urine samples of school students, according to data from **Table 1**, they were divided into three groups. Results for school children are presented in **Table 2**.

Table 2. *Content of thiocyanates in the urine samples of school children.*

Tested / Indicators	Nonsmokers	Smoking up to 10 cigarettes per day	Smoking more than 10 cigarettes per day
Number	13	47	26
Thiocyanates (X±SD) μmol/l	25,46 ± 6,19	62,78 ± 14,10	133,99 ± 31,64
Statistical reliability	p< 0,001	p< 0,001	

The results obtained after determination the quantity of the thiocyanates in urine samples and from the followed inquiry among the school children are presented on **Table 3**. As is seen on the **Table 3** there was no correlation between the values obtained for the thiocyanates in urine samples and data obtained by the inquire.

When the results obtained by the inquire and the spectrophotometrical method were compared considerable differences were stablished **(Fig 1**).

Based on the present study is evident that the smokers are more prevalent among school children. Data from the questionnaire method showed 18% are smokers and 82% are non-smokers. However, the measured levels of thiocyanates in the urine samples showed that 85% were smokers and 15% nonsmokers. In this high percentage of smokers may also include passive smoking, but nevertheless the data were very disturbing (38). Comparing the present results obtained from the mixed gender group of 15-18 year-olds school students, to the results of our formerly study with 16 years-old boys (25) was found that the number of smokers were increased by 10%.

Table 3. *Results obtained from the spectrophotometrical method and from the inquire. (presented in %)*

Indicators \ Tested	Nonsmokers	Smoking up to 10 cigarettes per day	Smoking more than 10 cigarettes per day
Number of schoolchildren according to the content of thiocyanates in urine (%)	13 (15,12)	48 (55,81)	25 (29,07)
Number of schoolchildren according to the inquire (%)	48 (55,81)	20 (23,26)	18 (20,93)

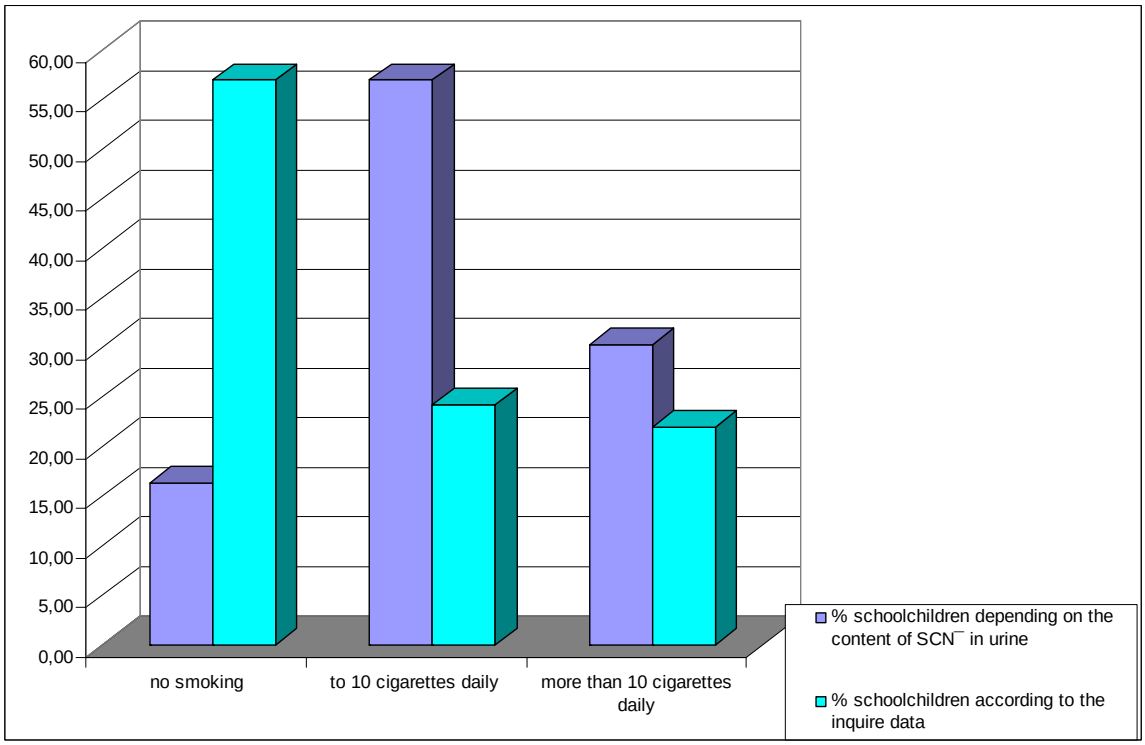

Fig. 1. Comparison of the data distribution in the % of schoolchildren in groups of smokers and non smokers

CONCLUSION

The data obtained shows that students from senior classes of schools are exposed to the adverse effects of smoking. Eighty-five percent of them smoke and about 30% smoked more than 10 cigarettes a day. These results for the concentration of thiocyanates in urine confirmed the seriousness of adolescent health and social problem of smoking. Those children, who started smoking at school age, will probably suffer the harmful effects of smoking - cardiovascular, pulmonary - including asthma, carcinogenesis, diabetes etc. This requires providing basic health education to school students to stop smoking and following adequate coherent effective controlled European legislation.

We also consider that our updated and computerized spectrophotometric method for determination of thiocyanates in urine samples as biomarkers for distinguishing smokers from

nonsmokers is suitable and also applicable for serial analyses.

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REFERENCES

1. Rytilä P., Kinnula V., Keuhkoahtaumataudin varhaisdiagnostiikka: keuhkofunktiotutkimuksista inflammaatiodiagnostiikkaan. *Duodecim* 121:2421–30. *(In Finnish),* 2005.
2. Pietinalho A., Pelkonen A., Rytilä P., Linkage between smoking and asthma. *Allergy* 64:1722–27, 2009.
3. Olds DL, Henderson CR, Jr., Tatelbaum R., Prevention of Intellectual Impairment in Children of Women Who Smoke Cigarettes During Pregnancy. *Pediatrics* 93:973-86, 1994.
4. Poswillo D, Alberman E., Effects *of* smoking *on the* fetus, neonate, *and* child. New York*: Oxford University Press,* p. *230*, 1992.
5. Peto R, Sarah D, Deo H, Silcocks P, Whitley E, Doll R, Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *British Medical Journal (BMJ)* 321(7257):323–29, 2000.
6. Ambrose JA, Barua RS, The pathophysiology of cigarette smoking and cardiovascular disease: An update. *J Am Coll Cardiol* 43:1731-37, 2004.
7. Lannerö E, Wickman M, van Hage M, Bergström A, Pershagen G, Nordvall L, Exposure to environmental tobacco smoke and sensitization in children. *Thorax* 63:172-76, 2008.
8. Halken S, Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. *Pediatr Allergy Immunol* 16:9-32, 2004.
9. Burr ML, Anderson HR, Austin JB, Respiratory symptoms and home environment in children: a national survey. *Thorax* 54:27-32, 1999.
10. Prescott SL, Effects of early cigarette smoke exposure on early immune development and respiratory disease. *Paediatr Respir Rev* 9:3-9, 2008.
11. Li JS, Peat JK, Xuan W, Berry G, Meta-analysis on the association between environmental tobacco smoke (ETS) exposure and the prevalence of lower respiratory tract infection in early childhood. *Pediatr Pulmonol* 27:5-13, 1999.
12. Gnoevyh VV, Smirnov AJ, Nagornov YS, Shalashov EA, Kupriyanov AA, Portnova Y, Bronchial asthma and smoking. *Pulmonology* 12:261-89, 2011.
13. Kumar R, Curtis LM, Khiani S, A community-based study of tobacco smoke exposure among inner-city children with asthma in Chicago. *J Allergy Clin Immunol* 122:754-59, 2008.
14. Benowitz NL, Jacob P, Ahijevych K, Hall S, LeHouezec J, Biochemical verification of tobacco use and cessation. *Nicotine Tob Reser* 4:149-59, 2002.
15. Prignot JJ, Recent Contributions of Air- and Biomarkers to the Control of Secondhand Smoke (SHS): A Review. *Int J Environ Res Public Health* 8(3):648-82, 2011.
16. Trullén AP, Bartolomé CB , Barrueco M, Herrero I, Jiménez CA, Nuevas perspectivas en el diagnóstico y evolución del consumo de tabaco: marcadores de exposición *Prevencion del tbaquismo* 8 (4), 164- 72, 2006.
17. Bacha ZA, Salameh P, Waked M, Saliva Cotinine and Exhaled Carbon Monoxide Levels in Natural Environment Waterpipe Smokers. *Inhalation Toxicology* 19, (9), 771-77, 2007.
18. Rendu F, Peoc'h K, Berlin I, Thomas D, Launay J-M, Smoking Related Diseases: The Central Role of Monoamine Oxidase. *Int. J. Environ. Res. Public Health 8*, 136-47, 2011.
19. Jarvis MJ, Sims M, Gilmore A, Mindell J, Impact of smoke-free legislation on children's exposure to secondhand smoke: cotinine data from the Health Survey for England. *Tob Control*, In Press, 2011.
20. Logue BA , Maserek WK, Baskin SI, The analysis of 2-amino-2-thiazoline-4-carboxylic acid in the plasma of smokers and non-smokers. *Toxicology Mechanisms and Methods* 19(3):202–08, 2009.
21. Khotsenko AA, Influence of smoking of mothers on the functional state of endothelium of children. *Medytsyna s'ohodni i zavtra* 4:99-101, 2008.

22. Branner B, Kutzer C, Wolfgang W Wickenpflug, Scherer G, Heller W, Richter E, Haemoglobin adducts from aromatic amines and tobacco specific nitrosamines in pregnant smoking and non smoking women. *Biomarkers* 3 (1):35-47, 1998.
23. Buratti M, Xaiz D, Caravelliand G, Colombi A, Validation of urinary thiocyanate as a biomarker of tobacco smoking. *Biomarkers* 2, (2):81-85, 1997.
24. Church T R, Anderson KE, Le C, Zhang Y, Kampa DM, Benoit AR, Yoder AR, Carmella SG, Hecht SS, Temporal stability of urinary and plasma biomarkers of tobacco smoke exposure among cigarette smokers. *Biomarkers* 15, (4), 345-52, 2010.
25. Angelova M, Aleksiev A, Mumdzhiev N, Nedkova V, Petrova CH, Rusanova R, Tiotsianatite kato kriteriĭ za razlichavane na pushachi ot nepushachi sred uchashtite se ot gorniya kurs. *Khigiena i zdraveopazvane*, 36 (1) 33 – 4, 1993.
26. Chattaraj S, Das AK**,** Indirect determination of thiocyanate in biological fluids using atomic absorption spectrometry. *Spectrochimica Acta* 47, (5):675-80, 1992.
27. Blount BC, Blasini LV, Analysis of erchlorate, thiocyanate, nitrate and iodide inhuman amniotic fluid using ion chromatography and electro spray tandem mass spectrometry. *Analytica Chimica Acta* 567 (1):87-93, 2006.
28. Xu J, Wang Y , Xian Y , Li H , Jin L , Tanaka K, Haraguchi H, Simultaneous determination of oxalate, thiosulfate, and thiocyanate in urine by ion-exclusion chromatography with electrochemical detection. *Chromatography* 56 (7-8), 449-53, 2002.
29. Michigami Y, Fujii K, Ueda K, Yamamoto Y, Determination of thiocyanate in human saliva and urine by ion chromatography. *Analyst* 117, 1855-858, 1992.
30. Toraño JS, van Kan HJM, Simultaneous determination of the tobacco smoke uptake parameters nicotine, cotinine and thiocyanate in urine, saliva and hair, is using gas chromatography-mass spectrometry for characterization of smoking status of recently exposed subjects. *Analyst* 128, 838-43, 2003.
31. Jafari MT, Javaheri M, Selective Method Based on Negative Electro spray Ionization Ion Mobility Spectrometry for Direct Analysis of Salivary Thiocyanate. *Anal. Chem. 82* (15), 6721–725, 2010.
32. Giraudi G, Grillo C, Direct spectrophotometric determination of thiocyanate in serum and urine with a continuous-flow analyzer. *Analytica Chimica Acta,* 128, 169-75, 1981.
33. Brauer V F H , Below1 H , Kramer1 A, Führer D, Paschke R, The role of thiocyanate in the etiology of goiter in an industrial metropolitan area. *European Journal of Endocrinology* 154, (2):229-35, 2006.
34. Gubina G, Vakulik G, Sbelyaev S, Vliyanie ottsovskogo kureniya na morfofunktsional′nye osobennosti platsenty. *Chim. Acta* 452: 295–302, 2008.
35. Demkowska I, Polkowska Ż, Kiełbratowska B, Namieśnik J, Application of ion chromatography for the determination of inorganic ions, especially thiocyanates in human saliva samples as biomarkers of environmentaltobacco smoke exposure. *Journal of Chromatography B* 875 (2): 419-26, 2008.
36. Swan GE, Parker SD, Cherney MA, Rosenman RH, Reducing the confounding effects of environment and diet on saliva thiocyanate values in exsmokers. *Addict Behav* 10: 187-90, 1985.
37. Galanti LM, Specificity of salivary thiocyanate as marker of cigarette smoking is no affected by alimentary sources. *Clin Chem* 43:184- 85, 1997. 38.
38. Mumdzhiev N, Ĭordanov I, Nedkova V, Angelova M. Tyutyunopusheneto i deteto. *Khigiena i zdraveopazvane* (4) 30 – 2, 1998.