Pre- and postnatal exposure of children to tobacco smoke during the first four years of life – observations of the authors

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Introduction

Environmental exposure to tobacco smoke is a significant threat for human health, where the higher is its degree, the more immature the human organism is. Therefore, the exposure to tobacco smoke in foetal life exerts unfavourable effects on developing foetus and may cause early and distant results in children.

Material and methods

The study comprised 318 children in their first four years of life, treated for various medical conditions. The examined children were divided into two groups, Group 1 – children exposed to Tobacco smoke – and Group 2 – a control group with children from non-smoking families. History data were obtained on the basis of a specially designed questionnaire, used by the doctor in an individual conversation with parent. In each third child from the group 1 cotinine concentration in urine was assayed by the method of high performance liquid chromatography-UV-VIS and the cotinine/creatinine ratio was calculated.

Results of study

Results demonstrated environmental exposure to tobacco smoke in 173 children (Group 1). Out of them 31.2% were the children whose mothers had smoked also during pregnancy (Subgroup A). The other 119 children from Group 1 were accounted to Subgroup B, i.e., children, where other household members had been smoking cigarettes. A comparative group comprised 143 children from non-smoking families. The results demonstrated then that 17% of all the examined children were those, exposed to tobacco smoke effects already in their foetal life, predisposing them to prematurity and low birth weight. Moreover, it was observed that the young age and lower education level of their parents, together with worse housing conditions, may suggest a predisposing character and role of the mentioned factors.

Key words

environmental exposure to tobacco smoke; children in pre- and postnatal period; cotinine

INTRODUCTION

Since the middle of the previous century, the cigarette smoking addiction has been very much widespread, as it has been demonstrated that more than a billion adults are addicted to tobacco products, while approximately 700 million children are passive smokers [1]. According to the World Health Organisation, Poland is in the group of countries with high tobacco consumption [2]. The results of the studies by Florek et al. [3] revealed 43% of men and 22% of women to be regular cigarette smokers. The results of similar studies, carried out in Lodz, demonstrated a high percent of exposure to tobacco smoke in 75.1% of boys in school age [4].

It should be emphasised that this exposure often begins as early as in the foetal life. The results of research, carried out by many authors, have demonstrated that approximately one third of pregnant women are tobacco smokers [5]. However, the incidence of smoking among pregnant women is differentiated in various countries, attaining the level of approximately 8% in Sweden [6], while being higher in other countries, e.g., 21% in the United States [7] and 57% in Portugal [8]. In Poland, the results of studies by Kalinka [9] have demonstrated 69.4% of pregnant women to be exposed to tobacco smoke and 20.8% to be active cigarette smokers.

Tobacco smoke contains a number of substances, toxic for health, such as nicotine, carbon monoxide, free radicals and other [10]. Cotinine, a major metabolite of nicotine, is metabolised in the liver and its blood serum concentration is strongly correlated with its urine levels [11]. Therefore, both passive and active tobacco smoking imposes very high risks for human health, the threat being inversely proportional to age [12, 13] thus being most detrimental for small children if they become passive smokers [1]. It has also been proven that the exposure intensity correlates with the daily number of smoked cigarettes at home, housing conditions – mostly density, i.e., the number of persons per one room [14]. Many authors have demonstrated that an exposure to tobacco smoke in prenatal period is particularly dangerous for developing foetus, exerting certain effects on offspring health status, increasing the risk of spontaneous abortion, premature deliveries and foetal hypotrophy [14]. Moreover, it may exert in children early and distant effects. In the youngest children, it may lead to infantile colic, what was demonstrated in a study by Milidou et al. [15], as the role of nicotine in the pathogenesis of colic is biologically...
AIM OF STUDY

The study aimed at an evaluation of pre- and postnatal exposure to tobacco smoke in children during the first years of life.

MATERIAL AND METHODS

The study comprised 318 children, treated at the 2nd Department of Paediatrics and Allergology of the Polish Mother’s Memorial Hospital – Research Institute – for various diseases during the years 2010–2011. The study group included 185 boys (58.2%) and 133 girls (41.8%), their age varying from 2 up to 48 months of life.

Each child was subject of enquiry for data, concerning the exposure to passive smoking at home environment, as well as the exposure to tobacco smoke during foetal life, using a specially designed questionnaire.

The questions also addressed the duration of pregnancy, birth weight and the Apgar score after birth, as well as the age and education of mother and father, the type of child’s nutrition in the infantile age, the daily number of smoked cigarettes and housing conditions, i.e., density = the number of persons per room and if the child has got its own room. All the data were obtained in an individual conversation with the child’s mother, father or foster parent/guardian.

Two groups were isolated among the study patients. The first group encompassed children exposed to tobacco smoke, further subdivided into two sub-groups:

A – children, the mothers of which smoked also during pregnancy;
B – children from homes where other household members smoked (e.g., father, grandmother, grandfather).

The second group included children from non-smoking families, which constituted a comparative, control group.

Cotinine and creatinine concentrations in urine were assayed in each third child, exposed to tobacco smoke.

An approval for the study was provided by the Commission of Research Ethics at the Polish Mother’s Memorial Hospital – Research Institute and a consent was obtained from the child’s parent or guardian.

Chemicals

Cotinine standard, creatinine standard and water for HPLC were purchased from Sigma-Aldrich (Warsaw, Poland), dichloromethane for HPLC, di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate from POCH S.A. (Gliwice, Poland). Acetonitrile for HPLC was purchased from J.T. Baker (Deventer, Netherlands) and ammonium hydrogen carbonate and sodium hydroxide from Chempur (Peccary Alsike, Poland).

Measuring apparatus

The LC system consisted of an Agilest Technologies (Palo Alto, USA) 1100 series HPLC system with degasser (model G1379A), is pump (model G1310A), UV-VIS detector (model G1314A) and injection valve (model G1375–87304). C18 column (4.6 mm x 150 mm, 2.6 mm particle size) (Kinetics, Phenomena) was used for the separation of cotinine and C18 column (4.6 mm x 150 mm, 5 mm particle size) (Agilest Technology) for the separation of creatinine.

Cotinine assay

A simple validated HPLC method for determination of levels of cotinine in urine of children was used. Urine samples were collected in polypropylene tubes and analyzed directly or kept frozen at -20 °C until analysis. 5 ml urine alkalinized by 500 ml 1M NaOH was added to a glass test tube, then 5 ml dichloromethane was added. Liquid-liquid extraction supported by ultrasounds was being made in an ultrasonic bath through 15 minutes. Organic phase was received and evaporated in a nitrogen stream. The dry residue was dissolved in 150 ml mixture of 95% 10 mmol NH₄HCO₃ and 5% CH₃CN. The mobile phase was composed for 10 mmol NH₄HCO₃ (phase A) and CH₃CN (phase B). The gradient elution was applied from 95% phase A and 5% phase B to 5% phase A and 95% phase B through 10 minutes, the composition of 5% phase A and 95% phase B was held by 2 minutes. The apparatus conditions were as follows: flow rate: 0.7 ml/min, wavelength: 254 nm, injection volume: 20 ml, run time: 12 minutes.

Creatinine assay

The results are expressed as ratio to the urinary creatinine concentration in mmol/mmol creatinine. Creatinine measurements in urine were carried out basing HPLC-UV-VIS method described previously by Kusnierek and co-authors (2006) with little modification in mobile phase composition.

Analysis ready sample was prepared by adding of 20 ml urine to 10 ml HPLC water. The mobile phase was composed of 98/2 phosphate buffer (20 mmol/L Na₂HPO₄, 3 mmol/L...
NaH$_2$PO$_4$) and acetonitrile (CH$_3$CN); the isocratic elution – 98/2 (v/v) was applied. The apparatus conditions were as follows: flow rate: 1ml/min, wavelength: 234 nm, run time: 3 min.

**STATISTICAL ANALYSIS**

Statistical calculations were carried out with support of the „STATISTICA” software package.

The main statistical parameters were calculated: the arithmetic mean, standard deviation and the coefficient of variation. The data were evaluated and confirmed as meeting the conditions of normal distribution. Differences between the groups were verified with Student’s t-test at the level of significance at $\alpha = 0.05$. The incidence of particular values was calculated for features in nominal scale. A comparison among the groups was performed by means of the chi-squared test.

**RESULTS**

An age analysis of the study group revealed the following ranking: infants – 189 (59.5%), children – 3–4 years of life (22.3%), children at the 2nd year of life – 58 (18.2%).

An evaluation of exposure to tobacco smoke demonstrated 173 (54.4%) children to be passive smokers (Group 1), including 54 children whose mothers used to smoke also in pregnancy (Subgroup 1). The other 119 children were included into Subgroup B, comprising the children from homes, where other household members smoked, e.g., father, grandmother, grandfather. The children, whose mothers smoked also during pregnancy (Subgroup A), constituted 31.2% of all the examined children, exposed to tobacco smoke, and 17% of all the study children. The second, comparative group comprised 145 children from non-smoking families. An analysis of sex distribution revealed that boys prevailed in all the studied groups.

See Table 1 for the numbers of children in particular study groups.

Following an analysis of perinatal data, 67 (21.1%) children were identified to have been born prematurely, i.e., between the 32nd and the 37th week of gestation, where their majority encompassed children, exposed to tobacco smoke.

An analysis of parent education demonstrated the highest prevalence of secondary (128 – 40.2%), then higher (82 – 25.8%) and vocational (47 – 14.8%) education among all the mothers vs. primary school education (26 – 8.2%) and lower education (8 – 2.5%). Similarly, there were more fathers below the 35th year of life in the group of children exposed to tobacco smoke (Fig. 2).

An analysis of parent education demonstrated the highest prevalence of secondary (131 – 41.2%) and higher (114 – 35.8%) education among all the mothers vs. primary school education (26 – 8.2%) and vocational (47 – 14.8%). Regarding the fathers, their majority reported secondary education (128 – 40.2%), then higher (82 – 25.8%) and vocational (81 – 25.5%). The least number of fathers had primary school education (27 – 8.5%).

Figure 3 presents in detail the education levels of the children’s mothers and fathers, with consideration of particular study groups. It was found that in the group of children exposed to tobacco smoke, the number of parents (both mothers and fathers) with primary and vocational education had been the highest, especially in the Subgroup of children with mothers smoking also during pregnancy. And reversely, in the comparative group (children from non-smoking families), there were significantly more mothers and fathers with higher education. Significant differences were also observed among the children, exposed to environmental tobacco smoke. There were more mothers with primary education among those, smoking also during pregnancy, while less with higher education, the differences being statistically significant. Similar, but not statistically significant differences were observed in fathers of the children.

In a subsequent stage of the study, nutrition types (natural vs. artificial), applied in the infantile age, became the subject of evaluation, see Fig. 4 for obtained results. Among 318 children, more than a half (58.8%) were breast-fed, with a significantly higher number of naturally fed children in the control group.

Table 2 presents housing conditions of the examined children. That analysis took into account the density index, i.e., the number of persons per room and the cases, where

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Similarly, birth weight evaluation disclosed 56 children with lower body mass (below 2,500 g), including significantly more children from the first group, i.e., passive smokers. Regarding that group, more children with low birth weight were found in Subgroup A, i.e., with mothers smoking also during pregnancy, the difference being, however, statistically insignificant, while no differences were identified in Apgar scores (Fig. 1).

An analysis of parent age demonstrated a prevalence of mothers below 30 years of life in the whole group of children (169 – 53.1%). Also, there were more fathers below the 35th year of life (203 – 63.9%). A detailed analysis of the parents’ age, taking into account particular study groups, demonstrated mothers below the 30th year of life prevailing in both study groups (Groups 1 and 2). However, that difference was most distinctive in Subgroup 1A, as mothers below 30 constituted almost 75% of the mothers smoking also during pregnancy. Similarly, there were more fathers below the 35th year of life in the group of children exposed to tobacco smoke (Fig. 2).

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<td><strong>Total</strong></td>
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*p < 0.05 – statistical correlation between (1;2, A:2)
Figure 1. The percent of children, born prematurely, with low birth weight and Apgar score > 4 < 7 among all examined children.

Figure 2. Age analysis of the children’s mothers and fathers.
1 Total number of exposed children (n=173)

1A Mothers smoking also in pregnancy (n=54)

1B Other smoking household members (n=119)

2 Non-exposed children (n=145)

* *p<0.05* between the exposed and non-exposed group for fathers and mothers, regarding primary, vocational and higher education.

**Figure 3.** Education analysis of the children’s mothers and fathers

**Figure 4.** The percent of breast-fed children in the infantile age
children had their own room. In the group of children from non-smoking families, the housing conditions had been better: lower density and the children having their own rooms. An evaluation of the daily number of smoked cigarettes in Group 1, revealed significantly more cigarettes smoked in Subgroup 1A, i.e., mothers who used to smoke also during pregnancy.

Regarding the group of children, exposed to tobacco smoke, in 52 (i.e. in 18 children from Subgroup 1A [mothers smoking also during pregnancy] and in 34 from Subgroup 1B [passive smokers]), the presence of cotinine and creatinine in urine was analysed and the cotinine/creatinine ratio was calculated, see Table 3 for obtained results.

Cotinine in urine was confirmed in all the examined children, while statistically significantly higher mean concentrations of cotinine and of the cotinine/creatinine ratio were assayed in the children from Subgroup 1A. It seems, however, that an interpretation of the obtained statistical difference should be careful because of the high variation ratio, which could have resulted from the differentiated time periods (1–3 days) of urine collection for cotinine assays.

## DISCUSSION

The reported study demonstrated a high (in >50% of the examined children) prevalence of environmental exposure to tobacco smoke. These results are compliant with reports of many authors who also demonstrate similar prevalence rates of passive smoking among children [4, 18]. The extent of this smoking culture authorises to perceive it as one of major threats for the health of both active and passive smokers [12].

The less mature organism and younger age, the higher are detrimental effects of toxic compounds in tobacco smoke. For this reason, there are many reports dedicated to the incidence and harmfulness of smoking by women in pregnancy. The first report on the issue was published in the 60-ties of the previous century, presenting the results of a study by Simpson, who demonstrated a higher percent of premature births by smoking women [21]. Many authors evaluate in their studies the frequency of cigarette smoking by pregnant women.

Coleman has been demonstrated that 8.1–30% of pregnant women all over the world are active smokers [22]. Other authors present a similar incidence with the average of 20–36% [5]. In our study, 17% of women smoked in pregnancy, resulting in as much as 32.1% of children, exposed to tobacco smoke. Our figures are close to the results of studies by Kalinka among pregnant women in Lodz [9].

The mechanisms of the detrimental effects of tobacco smoke have not yet been fully identified. Toxic substances, contained in tobacco smoke, including, among others, nicotine, carbon monoxide and cyanide permeate through the placenta into foetal circulation system [23]. Nicotine is responsible for shrinking of peripheral blood vessels and increasing of blood pressure, carbon monoxide binds with haemoglobin, forming carboxyhaemoglobin and cyanide plays its role in delayed foetal growth. It leads to reduced blood flow through the placenta, intrauterine foetal anoxia, villus hypertrophy and an impeded access to nutritional components [18, 23].

The other children, exposed to tobacco smoke, (Subgroup 1B) – passive smokers – are also sensitive to the effects of toxic compounds. It should be emphasised that, following the results of studies by Florek et al. [10], published in 2003, the amount of toxic compounds in exhaled tobacco smoke by an active smoker is higher than that, inhaled by the same smoker, e.g., the concentration of carbon monoxide may be even 5 times higher.

The results of studies by many authors have demonstrated that environment al exposure to the effects of tobacco smoke, both passive and active, in pregnant women may lead to foetal anoxia and, in consequence, also to foetal malnutrition [18]. It increases the incidence of premature deliveries and/or decreased birth weight in delivered children. In our study, each fifth child was prematurely born, with a prevailing, statistically significant number of the children having been exposed to tobacco smoke, what is compatible with observations of many authors [9]. Similarly, there were more children, although not statistically significantly, with lower birth weight among the examined patients, exposed to tobacco smoke, especially when their mothers smoked also during pregnancy. These findings are compatible with those of other authors [18, 24].

An age analysis of the parents of those children, whose mothers also smoked during pregnancy, revealed the highest percent of mothers below the 30th year and fathers below the 35th year, what coincides with the observations of other authors [4]. Both our observations and the results of other authors indicate a need of a broad education on the detrimental, harmful effects of environment al exposure to tobacco smoke, exerted on the health condition of parents and their children, even with very distant effects. It seems that the education should be started as early as in the kindergarten age. Similarly, the analysis of parent education demonstrated considerable differences. There were significantly more mothers and fathers with lower education among the children exposed to tobacco smoke, most distinctive among the children, whose mothers smoked also in pregnancy. The results are also compatible with the observations of other authors, confirming the need of broad antinicotinic education.

In the medical literature, the effect of housing conditions is also emphasised. The obtained results are compatible with those of other authors, indicating in a similar way a possible role of housing conditions in the evaluation of exposure to tobacco smoke [1, 18].

### Table 3. Concentrations of cotinine and values of the cotinine/creatinine ratio in children of both subgroups exposed to tobacco smoke

<table>
<thead>
<tr>
<th>Children exposed to tobacco smoke</th>
<th>Cotinine concentration in urine (nmol/ml)</th>
<th>Cotinine/creatinine ratio (umol/mmol)</th>
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<tr>
<td></td>
<td>n</td>
<td>SD</td>
</tr>
<tr>
<td>A. mothers smoking also in pregnancy (n=54)</td>
<td>18</td>
<td>108.54</td>
</tr>
<tr>
<td>B. other smoking household members (n=119)</td>
<td>34</td>
<td>48.02</td>
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Student’s t-test (p<0.05)

<table>
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<tr>
<th>n</th>
<th>0.03</th>
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<tr>
<td>Total</td>
<td>70.22 +/-114.84</td>
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Reports from the recent years indicate that in mothers, who smoke and breast-feed their children, the toxic compounds from tobacco smoke, pass into mother’s milk [14]. Among the examined children, there were more naturally fed children in the control group (64.1%) than in the group of children exposed to tobacco smoke (54.3%), however, in the exposed group, the children from smoking mothers were more frequently breast-fed. The differences were not statistically significant. It should be emphasised that the results of Nishijo et al. [25] indicate that human milk of smoking mothers contains elevated levels of heavy metals, especially cadmium, which affects the metabolism of microelements, e.g., selenium, zinc, copper and other, compromising their bioavailability, what may affect the enzymatic anti-oxidative barrier and support the development of infectious diseases. It may also predispose to certain allergic diseases. Therefore, educational activities should be undertaken in case of every smoking pregnant and then breast-feeding woman about possible threats for her child.

CONCLUSIONS

1. The study demonstrated 54.4% of the examined children to have been exposed to tobacco smoke at home environment during the first four years of life (passive smokers).
2. One third of the passive smokers were exposed to tobacco smoke effects already in the prenatal period.
3. Cigarette smoking by mothers of the examined children during pregnancy may also predispose the children to prematurity and low birth weight.
4. Young age and lower education level of parents, together with worse housing conditions, more often confirmed among the passive smokers, may be suggestive of their predisposing character.
5. The obtained results indicate a need of a broad anti-nicotine education in various age groups.

REFERENCES