The Influence of Smoking on Nicotine Exposure Biomarkers and Inflammatory Profile Among Foster Care Teenagers, Romania

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Foster care young people have an increased overall risk for the development of chronic health conditions. Smoking is a major risk factor for many conditions with inflammatory component. We performed a cross-sectional pilot study to assess the correlation between tobacco consumption status and inflammatory profile among adolescents living with foster parents in the central region of Romania. A number of 35 teenagers aged 10-18 were enrolled. Blood samples were collected by venipuncture for complete blood count (CBC), fibrinogen, Interleukin-6 (IL-6), C reactive protein (CRP). Exhaled CO was measured and urine samples were collected in the same session for cotinine assessment. Of the 123 teenagers initially enrolled, 35 completed the entire study protocol. Urinary cotinine accurately reflected smoker status. Smoking did not affect hemoglobin levels or other hematological parameters in foster care teenagers. The value of C-reactive protein was higher in smokers. Plasma fibrinogen correlated with the daily number of cigarettes. Interleukin-6 did not correlate with the smoker status or the daily number of cigarettes consumed. Our study highlights the existence of an inflammatory response to smoking in foster care adolescents, a social category otherwise predisposed to various addictive behaviors.

Keywords: foster care teenagers, smoking, inflammatory profile, biomarkers, prevention

Foster care young people have both increased cardiovascular risk and overall risk for the development of chronic health conditions [1]. Nevertheless, smoking is a major risk factor for many conditions with inflammatory component (chronic obstructive pulmonary diseases, cardiac diseases) [2].

In smokers, certain biomarkers, such as increase of leukocytes, IL-1β, IL-6, TNF-α, C-reactive protein (CRP), and fibrinogen, indicate the reactivity of the body to the toxic effects of cigarette smoke components and indicate subclinical changes that may be the basis for subsequent complications [3,4]. The mechanism by which smoking induces inflammatory changes should be interpreted both by acute and chronic exposure to cigarette smoking. Acute effects occur both in occasional smokers and, light smokers and, inflammation progression becomes more important as the exposure to smoking extends [5, 6].

Nicotine, the alkaloid, is found in nature and acts as a botanical insecticide in tobacco leaves. The alkaloid contributes to addiction among cigarette smokers. The serum concentration of nicotine rise quickly during a smoke inhalation event and peak at the completion of the event. Nicotine is metabolized in liver by Cytochrome P450 2A6 to cotinine. Approximately 70-80% of nicotine is converted to cotinine, which is its primary metabolite. Cotinine, a predominant metabolite of nicotine from cigarettes, is now used as a biomarker for both active use of tobacco and for secondhand smoke exposure (fig. 1). Cotinine has been measured in a variety of biological fluids and tissues including plasma, serum, urine, hair and nails, with the longest half-life measured in serum.

Since young people in foster care system appear to be at increased risk for various chronic diseases [1], we aimed to evaluate whether smoker status was associated with an inflammatory response as an additional potential risk factor. This may help us gain insight on the pathophysiological processes associated with smoking in these young individuals.

Fig. 1. Primary pathways of nicotine oxidation

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Experimental part

Method

We performed a cross-sectional study to assess the correlation between tobacco consumption status and inflammatory profile among adolescents living with foster parents in central region of Romania. The study was conducted from February till May 2017 at the General Directorate for Social Assistance and Child Protection in Mures and Cluj. The Ethics Committee of the Romanian Society of Pneumology approved the survey in accordance with the national and European law. It followed the current regulations on the protection of personal data. The study also received the approval (No. 443/Nov 2016) of the Ethics Committee of Iuliu Hatieganu University of Medicine and Pharmacy in Cluj Napoca, Romania.

Each participant expressed their consent for the biohumoral sampling. The consent was signed both by the legal guardian and the Director of the General Directorate for the Protection of the Child, Mures.

Selection criteria

The study initially enrolled 123 foster care teenagers aged 10-18 years, smokers or never smokers. They were informed on the voluntary nature of their participation and were reassured on the anonymous character of data handling.

Individuals who did not express their consent or for whom their legal guardian did not agree with the participation in the study, were excluded, therefore the study group reached 87 subjects. Individuals who declared to be on chronic treatments or suffered from acute illnesses were also excluded. Fifty-four participants who met all criteria, expressed their acceptance for participation, and were able to read and fill in the questionnaires, were finally enrolled. Thirty-five teenagers presented at the scheduled date for bio-humoral sampling; the rest did no longer answer the invitations. In our study, we analyzed the data from this final lot.

Participants were also asked to complete a questionnaire containing demographic data (name, sex, age) and 6 questions about smoking. The Fagerström score adapted to adolescents was calculated in smokers to assess the level of nicotine addiction [7,8]. Affirmative smokers also completed a questionnaire for the Fagerström test adapted to adolescents, which includes 7 items scored between 0 and 2. The maximum score that can be obtained for the questionnaire is 9 [9]. All questionnaires were self-reported (table 1).

The legal guardians were not present in the same room with the participant during questionnaire completion, CO assessment and biological sample collection in order not to influence the results. Exhaled CO was assessed using a portable analyzer (Micro Smokerlyzer, Bedfont Scientific, England). The results were evaluated according to the manufacturer's recommendations. CO levels were self-reported (table 1). All questionnaires were self-reported (table 1).

The confidentiality of the participants was respected. The samples were destroyed after processing, according to the protocol signed between the investigators and the laboratory that processed them, thus respecting the Ethics Committee's agreement.

Biochemical analyses

Blood samples were collected by venipuncture for complete blood count (CBC), fibrinogen, IL 6, C reactive protein (CRP). Urine samples were collected during the same session, in labeled sterile containers.

All biological samples were sent to the laboratory within 24 hr. The CBC was performed within the collection day. Plasma fibrinogen was assessed by the Clauss coagulation method. Serum CRP was assessed by a turbidimetric method with a detection limit of 0.302 mg/dL. IL-6 was measured in serum by electrohemiluminescence with a Cobas Core® immunological automated analyzer (Roche Diagnostic Systems), which enables single IL-6 measurement within about 1 hour. The detection limit of the assay was 1.5 pg/mL.

Cotinine in urine samples was assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The reference values for urine cotinine were: lower than 50µg/L for non-smokers, lower than 100µg/L for passive smokers and more than 250µg/L for smokers.

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Statistical analyses

Data were centralized in a Microsoft Excel spreadsheet and subsequently analyzed in the R statistical environment. Two-sample comparisons of numeric data were performed using Wilcoxon’s rank sum test. Association between paired samples was assessed using Spearman’s rho. Categorical variables were compared with Fisher’s exact test. Quantitative variables were compared with Kruskal-Wallis test for rank sums. The threshold for statistical significance was set at 0.05. The reported p-values are two-sided.

Results and discussions

Twenty-eight percent of the participants (35/123) initially enrolled completed all the phases of the study. The average age of the participants was 13.37 years, 45.7% were males and 54.3% were females. The percentage of smokers (daily and intermittent) among study participants...
was 25.7%. In the smokers group, 36.4% started smoking between 10-14 years. Among smokers, 23.8% smoked between 1-5 cigarettes/day. Forty percent of foster parents were smokers, (table 2).

In smokers who completed the Fagerström score, the average value was 4, demonstrating a mild-to-moderate addiction. The average value of exhaled CO (in all participants) was 1.24 ppm (parts per million). Carbon monoxide in the exhaled air was higher in smokers than in non-smoker adolescents (p = 0.001), (fig. 2A). In smokers, the average urinary cotinine was 1639 µg/L, with a range of 20-3452 µg/L (table 3).

Urine cotinine accurately reflected smoker status (p <0.001). Moreover, its level significantly correlated with the average daily number of cigarettes smoked by children (rho = 0.75, p <0.001) (fig. 2B). Smoker status was associated with more advanced age (p=0.02).

| Parameter | Value n, % |
|-----------|------------|
| Age(years) | 12.3 (2.37) |
| Sex, male | 16 (45.7) |
| Smokers (daily and intermittent) | 9 (25.7) |
| Age of onset smoking, years | |
| - Never smoking | 23 (65.8%) |
| - < 8 | 2 (5.7%) |
| - 8-10 | 2 (5.7%) |
| - 10-12 | 4 (11.4%) |
| - 12-14 | 4 (11.4%) |
| - >15 | 0 (0%) |
| Cigarettes/day | |
| - 0 | 26 (74.2%) |
| - 1-5 | 5 (14.5%) |
| - 6-9 | 1 (2.8%) |
| - 10-19 | 0 (0%) |
| - >20 | 3 (11.65%) |
| Foster care minors with smokers foster parents | 14 (40) |

Although smoker status in adolescents was not associated with the smoker status of their foster parents (p = 0.432), the number of cigarettes consumed daily was higher in those with smoker foster parents (p = 0.043).

Smoking did not affect hemoglobin levels or other hematological parameters in foster care teenagers. In the studied group, the hemoglobin level did not correlate with the carbon monoxide in the exhaled air (p = 0.25).

Among the inflammatory markers studied, CRP was higher in smokers (p = 0.007) and showed moderate correlation with the average number of cigarettes consumed daily (rho = 0.46) (fig. 2). In smokers, plasma fibrinogen was correlated with the daily number of cigarettes (rho = 0.76, p = 0.047). Due to the relatively small number of participants in this pilot study, we could not emphasize any association between plasma IL-6 and smoker status or the daily number of cigarettes consumed (p = 0.174).

Table 2
SOCIO-DEMOGRAPHIC CHARACTERISTICS OF FOSTER CARE TEENAGERS IN CENTRAL REGION OF ROMANIA, 2017

Table 3
VALUES OBTAINED IN THE STUDY GROUP-FOSTER CARE TEENAGERS, IN CENTRAL REGION OF ROMANIA, 2017

| Parameter | Value |
|-----------|-------|
| Fagerström score (x=5) | 5 (1-7) |
| CO exhaled, ppm | 1.24 (1.20) |
| Hemoglobin, g/dL (12-15.4 g/dl) | 13.71 (1.34) |
| Platelets, x10^12/L (160000-385000/ mmnc) | 258 (51) |
| Leukocytes, x10^9/L (4200-10800/ mmnc) | 7.94 (1.74) |
| Eosinophils, % (0.5-5.5%) | 2.98 (2.20) |
| CRP, mg/L=0.33 mg/L | 0.37 (0.27) |
| Fibrinogen, g/dL (1.8-3.5 g/dl) | 2.32 (0.39) |
| IL-6, pg/mL (<7 pg/ml) | 2.01 (1.52) |
| Urinary cotinine, µg/L | 390 (847) |

Values are presented as average (standard deviation), except when another form is specified. *Median (interval)
In this cross-sectional study, we aimed to establish whether there is a correlation between the smoker status of foster care teenagers and their inflammatory profile, considered a risk factor for various medical conditions. We found evidence of an inflammatory response in smokers, reflected by the correlation of CRP and fibrinogen with the number of cigarettes consumed daily by these teenagers.

Biomarkers such as CO and cotinine (a nicotine metabolite) are used in tobacco cessation studies to assess smoking status [9]. There is a dose-dependent relationship between smoking and the levels of urinary cotinine and exhaled CO [10]. Exhaled CO and COHb (in blood) are highly correlated. The systemic inflammatory response triggered by smoking, involved in the onset and progression of respiratory diseases, results in increased cardiovascular morbidity and mortality through the activation of vascular endothelium, leading to aggravation and instability of the atherosclerotic plaque at adult age [3]. At the age of adolescence, occasional smoking is commonly encountered, and even daily smokers consume a small number of cigarettes per day [11]. Therefore, CO and cotinine levels observed by us were not very high, confirming that our participants were light and intermittent smokers.

Cotinine, a predominant metabolite of nicotine from cigarettes, is now used as a biomarker for exposure to cigarette smoke [12]. Due to a long half-life (approximately 20 h), urinary cotinine concentration is a valuable indicator of tobacco smoke exposure [13]. Cotinine levels after exposure to cigarette smoke are similar in intermittent smokers and daily smokers [14].

Inflammation caused by smoking can lead to an increased production of cytokines such as IL-1, IL-6, TNFα, which upregulates the production of CRP in hepatocytes. CRP is an acute phase reactant that can be considered a predictor of future cardiovascular risk [15]. Existing data suggested a correlation between adult smoker status and C-reactive protein (CRP), leukocyte count, IL 6, IL 1β, and TNF α [5]. Statistically significant correlations were observed in our study between smoking and serum CRP, fibrinogen, urinary cotinine and exhaled CO. Based on the observation that there is a correlation between CRP and adult smoker status, another study demonstrated that this correlation also holds true in adolescents. CRP levels in smoker adolescents being significantly higher than in non-smokers of similar age [17]. CRP levels were also increased in adolescents exposed to passive smoking [16]. Also, a correlation between the number of daily smoked cigarettes and the level of CRP has been demonstrated [17], correlation also sustained by our study results.

Exposure to passive smoking in the family is definitely an additional health risk factor, both by the developing inflammation due to the compounds resulting from passive smoking, and by increasing the risk of adolescents of becoming active smokers [18]. Although not statistically significant, the daily number of cigarettes was higher in foster care adolescents with smoker foster parents.

Non-correlation of hematological parameters with smoker status can be attributed to the short smoking history in this age group, most of smoker participants being occasional smokers or light smokers. The direct correlation between increased tobacco consumption and inflammation is stronger in daily smokers than in former smokers, thus active smoking prevention and smoking cessation measures need to be augmented, especially in disadvantaged social categories bearing additional risk factors [18]. Developing a large education in general population by the instrumentality of the health personnel and by implementing antismoking policy initiatives could help further reduction of the diseases burden caused by smoking [19].

One of the study limitations is the small number of participants. Based on these results, we plan to continue our investigation in a multicentric study. The particularity of this research is the special social status of these teenagers. Institutionalized minors from families with a poor medical, cultural and socio-economic situation often have multiple associated diseases [7, 20]. The determination of risks in this particular social category has been a strong point of this research. Our study underlines the existence of an inflammatory response to smoking in foster care adolescents, a social category otherwise predisposed to various addictive behaviors. This suggests a higher risk for the development of smoking-related complications in these individuals and warrants further investigation of the clinical impact of these findings. The smokers had poor or moderate motivation for smoking cessation [21].

Training the future health promoters is a priority and may be an issue for a smoke-free behavior in general population [22].

**Conclusions**

Our study highlights the existence of an inflammatory response to smoking in foster care adolescents, a social category otherwise predisposed to various addictive behaviors.

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