Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 41 (2008) 152–161
CLINICAL BIOCHEMISTRY

Blood levels of polycyclic aromatic hydrocarbons in children and their association with oxidative stress indices: An Indian perspective

Vipul K. Singh [a], D.K. Patel [b,*], Jyoti [a], S. Ram [d], N. Mathur [c], M.K.J. Siddiqui [a]

[a] *Analytical Toxicology Section, Industrial Toxicology Research Centre P.O. Box 80, M.G. Marg, Lucknow 226001, India*
[b] *Analytical Chemistry Section, Industrial Toxicology Research Centre P.O. Box 80, M.G. Marg, Lucknow 226001, India*
[c] *Epidemiology Section, Industrial Toxicology Research Centre P.O. Box 80, M.G. Marg, Lucknow 226001, India*
[d] *Department of Pediatrics, King George's Medical University, Lucknow 226001, India*

Received 3 May 2007; received in revised form 27 October 2007; accepted 22 November 2007
Available online 5 December 2007

Abstract

Background: Polycyclic aromatic hydrocarbons (PAHs), some ubiquitous environmental contaminants are capable to cause oxidative stress, during its metabolism. It is believed that many diseases that have a common origin in oxidative stress begin in childhood. Considering oxidative stress evolved during PAHs metabolism as one main mechanism responsible for health hazards related to PAHs exposure in children, we biomonitored blood PAHs levels in connection with redox status among children of Lucknow (India).

Methods: The study consisted of children ($n = 50$) who visited to the Pediatrics Department (KGMU) Lucknow for usual health check-up camp over the study period (August 2005–July 2006). Blood samples were drawn and levels of acenaphthylene, anthracene, phenanthrene, fluoranthene, naphthalene, pyrene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*a*)pyrene were determined by HPLC–FD/UV. Malondialdehyde (MDA), glutathione (GSH) levels, catalase (CAT) and superoxide dismutase (SOD) activity were also determined to evaluate redox status.

Results: Significantly elevated carcinogenic blood PAHs levels (125.55 ± 26.99ppb, $p < 0.05$) were found in rural children compared to children from urban region (23.96 ± 13.46). Results revealed that remoteness between residence and highway/traffic, significantly influences the blood levels of carcinogenic PAHs. There were significant correlations between total PAHs and MDA ($r = 0.82$, $p < 0.001$), carcinogenic PAHs and SOD activity ($r = 0.35$, $p < 0.01$), Total PAHs and blood GSH level ($r = -0.49$, $p < 0.01$) and carcinogenic PAHs and CAT activity ($r = 0.42$, $p < 0.01$). Blood MDA level was also found correlated with increasing body mass index (BMI) ($r = 0.29$, $p < 0.05$).

Conclusion: Our results showed blood PAHs levels in children significantly correlated with oxidative stress and altered antioxidant status. It supports our hypothesis that the children exposed to high PAHs level will suffer more to oxidative stress that may lead to possible health risks. Additional studies with large sample size are considered necessary to strengthen the database and also to explore the PAHs associated health risks in children.

Keywords: Children; Polycyclic aromatic hydrocarbons; Oxidative stress

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds with two or more fused benzene rings produced by incomplete combustion of organic substances involved in natural and anthropogenic processes. It has been well established that PAHs have carcinogenic, mutagenic and teratogenic effects [1–3]. Exposure to these compounds is a public health concern particularly in children, who are one of the most susceptible groups of the population. In many cases, children are thought to have greater exposure to airborne pollution per body weight than adults, because children generally tend to spend more time indoors, have higher physical activity and have a higher ventilation rate than adults [4,5]. Thus, they are exposed proportionally to higher doses of the toxic compounds [6,7]. Moreover, exposure to genotoxic carcinogenic compounds at a young age may represent a health risk, i.e. by causing genetic damage (mutation, sister chromatid exchanges and other genetic disruption) [8–10] that may increase the risk of cancer later in life [2,11]. Epidemiological and experimental data reported increased cancer risks following

* Corresponding author. Scientist-C, Analytical Chemistry Section, Industrial Toxicology Research Centre, P.O. Box 80, M.G. Marg, Lucknow 226001, India.
E-mail address: vipulitrc@rediffmail.com (D.K. Patel).

0009-9120/$ - see front matter © 2007 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2007.11.017

childhood exposure to carcinogens as compared with exposure occurring at a mature age [12]. Air and water monitoring of some of the major Indian cities showed high PAHs levels in urban environment; ambient air levels in Delhi ranged between 9.4 and 60.9ng/m^3, Kolkata 22.91–190.96ng/m^3, while in Lucknow water levels of PAHs were ranged 0.04–65.85μg/L [13,14], which are higher than the standard limits of 5ng/m^3 Central Pollution Control Board (CPCB, India) and 0.2μg/L of Bureau of Indian Standards (BIS, India) respectively [15,16]. Considering elevated levels of PAHs in Indian environment and its possible relation with children health, sufficient interest was generated to bio-monitor blood PAHs levels in children and their associated biological effects.

Several reports identify certain PAHs which clearly resemble steroid hormones as example of environmental estrogens [17] and human exposure to non-steroidal environmental estrogens is thought to be a risk factor for endocrine disruption and development of cancer [18]. Immunosuppression due to PAHs may also indirectly contribute to their carcinogenic properties but remain incompletely understood because most of the published studies have been performed only on mice [19]. Besides these pathways, induction of oxidative stress has been proposed previously as a possible mechanism of action for carcinogenesis [20]. Also, it is believed that many diseases that have a common origin in oxidative stress begin in childhood [21]. PAHs are reported to disturb the antioxidant defense system and responsible to induce oxidative stress. It is well known that, PAHs are not known to exhibit acute symptoms; metabolic activation of PAHs by cytochrome P 450 (CYP) 1A1-catalyzed reactions generates electrophilic metabolites and other reactive oxygen species (ROS), which tends to bind covalently with DNA and also cause interference with cell homeostasis [22,23]. Increased ROS generation or state of oxidative stress have been shown to be linked with a lot of pathophysiological changes in children like asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, juvenile rheumatoid arthritis, cholestatic liver diseases and diarrheal diseases [24–31]. We hypothesized that children more exposed to PAHs will suffer to more oxidative stress that may lead to health risks. So oxidative stress generated during PAHs metabolism may be one of the main mechanisms responsible for developing health risks among PAHs exposed children (Fig. 1). Therefore in order to bio-monitor blood PAHs levels and associated redox status among the children of Lucknow City (India) present study was designed. The purpose of our research was (i) to estimate total blood burden of PAHs and (ii) to elucidate the influence of multimedia PAHs exposure on oxidative stress level, as early biological effects leading to health hazards and antioxidant defense system of children, who are exposed non-occupationally only through general environment.

Material and methods

Study group

50 healthy children (2–10years), those presented at Department of Paediatrics, King George's Medical University (KGMU), Lucknow, India over the study period of one year (August 2005–July 2006) for routine health check-up, were selected for the study. This medical university is a referral centre for the treatment of several chronic diseases of children from Lucknow, the capital of the most populous state of Uttar Pradesh, India. The objective and aim of the study were described to the parents/guardians of all participants and their consents were taken for this particular study. Additionally institutional ethics committee clearance was also obtained for collecting the blood samples. All the participants were from general population of in and around Lucknow and adjoining rural areas.

All the children participated in the study fulfill the inclusion criteria of "normal blood profile" i.e. hemoglobin concentration (> 12g/dL), hematocrit (40–47%), red blood cells count (4.0–5.5 × 10^{12}/L), white blood cells count (4.0–11.0 × 10^9/L), platelets count (200–500 × 10^9/L) and not suffering from any chronic disease(s). Trained physician or nurses interviewed children and their parents to obtain data regarding age, gender, height, weight, and body mass index (BMI), socioeconomic status (SES), area of residence, relevant medical history and any history of exposure to chemicals. Other relevant information of known PAHs sources like type of kitchen fuel use, cooking place in home, smoking habit of the parents/family members and distance of residence from highway was also included in the questionnaire. None of the subjects reported any occupational/accidental exposure to known PAHs sources, and therefore, the main source of the detected PAHs level is expected to be through ambient air/food chain contamination, water and soil/dust. We adopted a set of five questions to assess environment tobacco smoke (ETS) home exposure among children, which was previously used by Willers et al. [32] in his study. Following questions were asked to parents/guardian at the time of interview:

1. Does anyone who lives in your home smoke cigarettes/bidi[1]?
2. If yes, how many people smoke inside your home?
3. Do you smoke cigarettes/bidi?
4. On average, how many cigarettes/bidi do you smoke a day?
5. Do you smoke inside your home?

The first question was used to identify ETS-free status; if no one who lived in the home smoked (including the parents/family members), the home was classified as "ETS absent." If the parents reported that they smoked cigarettes (question 3) in their home (question 5), the homes were classified as "ETS present".

Sample collection and processing

All the study samples were collected at the time of enrollment in the Department of Paediatrics (KGMU) Lucknow. Approximately 3.0mL of venous blood was withdrawn from each

[1] Bidis are small, brown, hand-rolled cigarettes made in India and some Southeast Asian countries. They consist of tobacco of sub standard quality, which is wrapped in a leaf from a tendu or temburni plant (temburini; *Diospyros melonoxylon*) and tied with a string at one end.