Short communication. Effect of polycyclic aromatic hydrocarbons on some clinical parameters in case of patients with lung cancer and associated diagnostics

Efectul hidrocarburilor policiclice aromatice asupra unor parametri clinici la pacienţi cu cancer pulmonar şi alte afecţiuni asociate

Mona E. Cioroiu[1,2*], Doina Tarcău[3], Simona Cucu Man[2], Bogdan I. Cioroiu[4], Raluca Mocanu[2], Irina Chisăliţă[5]

1. Department of Clinical Biochemistry, Clinical Hospital of Pneumology Iasi, Romania
2. Department of Chemistry, "Al. I. Cuza" University of Iasi, Romania
3. Water Quality Laboratory, Prut Water Branch, Iasi,Romania
4. Department for Quality of Drugs, "Gr.T.Popa" University of Medicine and Pharmacy, Iasi, Romania
5. Department of Anatomical Pathology, Clinical Hospital of Pneumology Iasi, Romania

Abstract

Tobacco smoke and environmental pollutants represent the first risk factors in case of lung cancer. The aim of this study was to investigate associations between polycyclic aromatic hydrocarbons (PAHs) levels in human lung tissue and modifications of serum and blood clinical parameters in patients with pulmonary cancer and associated diagnostics such as diabetes, HTN and anemia. This study included 31 histologically confirmed lung cancer cases diagnosed consecutively in the Clinical Hospital of Pneumology Iasi from 2008 to 2009. Analyses were carried out using accelerated solvent extraction technique and HPLC with a fluorescence detector. Higher concentrations of non carcinogenic PAHs (ΣPAHs =79.96 ng/g wet tissue) and carcinogenic PAHs (ΣPAHs = 7.72 ng/g wet tissue) were found in patients with HTN as secondary diagnosis, followed by patients with diabetes (non carcinogenic ΣPAHs = 57.71 ng/g wet tissue, carcinogenic ΣPAHs =3.18 ng/g wet tissue) and anemia (non carcinogenic ΣPAHs = 32.47 ng/g wet tissue, carcinogenic ΣPAHs = 5.86 ng/g wet tissue). RBC, HCT and HGB values were low for 65% of the investigated subjects, and also were positively correlated ($p<0.05$) with levels of benzo(g,h,i)perylene in lung tissue. Serum C reactive protein and plasma fibrinogen exceed the reference values proposed by the clinical laboratory and were positively correlated with benzo(a)anthracene and fluorene.

Keywords: *Pollution, lung cancer, BaPy, RBC, CRP*

Rezumat

Fumatul şi poluanţii din mediul reprezintă principalii factori de risc în cazul cancerului pulmonar. Scopul studiului a fost evaluarea corelaţiilor dintre concentraţiile de hidrocarburi policiclice aromatice (PAHs) în

[*]**Corresponding author:** mona.cioroiu@ymail.com, Tel+40-232-201063., Fax.+40-232-201313

ţesut pulmonar şi modificarea parametrilor clinici în cazul pacienţilor diagnosticaţi cu cancer şi alte diagnostice secundare, cum ar fi: diabet, HTA şi anemie. În studiu au fost examinaţi 31 de pacienţi diagnosticaţi cu cancer pulmonar la Spitalul Clinic de Pneumologie din Iaşi în anul 2008-2009. Analizele au fost realizate utilizând extracţia accelerată cu solvent şi HPLC cu detector de fluorescenţă. Concentraţii mari de PAHs necancerigene (ΣPAHs =79.96 ng/g ţesut) şi cancerigene (ΣPAHs = 7.72 ng/g ţesut) au fost determinate în cazul pacienţilor având HTA ca diagnostic secundar, urmate de pacienţi cu diabet (ΣPAHs necancerigene = 57.71 ng/g ţesut, ΣPAHs cancerigene =3.18 ng/g ţesut) şi anemie (ΣPAHs necancerigene = 32.47 ng/g ţesut, ΣPAHs cancerigene = 5.86 ng/g ţesut). RBC, HCT şi HGB prezintă valori scăzute pentru 65% din subiecţii investigaţi şi au fost corelate pozitiv ($p<0.05$) cu concentraţiile de benzo(g,h,i)perilen din ţesut pulmonar. Proteina C-reactivă şi fibrinogenul depăşesc intervalul de valori propus de laborator şi au fost pozitiv corelate cu benzo(a)antracen şi fluoren.

Cuvinte-cheie: *Poluare, cancer pulmonar, Benzo-a-piren, RBC, CRP*

Introduction

PAHs are compounds with two or more fused benzene rings produced by incomplete combustion of organics substances involved in natural and anthropogenic processes (1). Polluted urban air and cigarette smoke are recognized as the two preeminent environmental sources of the potential carcinogenic stimuli (2) Tobacco is probably the most significant source of PAHs exposure in humans. It was estimated that a smoker is exposed to about 1-30 µg/day/pack of cigarettes (3). Industries, vehicular traffic and domestic burning are major contributors to pollution in Romania. It has been already reported that environmental levels of PAHs in Romania are much higher than the standards limits prescribed by government regulatory agencies (4). In recent years Romania has had a national implementation plan for reducing PAHs under the Stockholm Convention (5).

According to different health and environmental protection organizations, such as the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (USEPA), several PAHs have mutagenic, carcinogenic and endocrine disrupting properties (6, 7, 8). Among the toxic compounds contained in cigarette smoke, which is known as the main risk factor for lung cancer, benzo-a-pyrene (BaPy) is well known as one of the most potent carcinogenic PAHs (9), also involved in other cancer types including skin, head and neck, bladder, and colon (10). The carcinogenicity of PAH compounds is mediated by DNA damage (11). The best-characterized pathway for genotoxicity of PAHs is represented by the covalent binding of the metabolically activated carcinogens to DNA bases to form DNA adducts (12). Investigating the significance of specific PAHs from air pollution related to lung cancer is very difficult. This situation is sustainable because the environmental exposures are relatively low, but it is important to consider the other sources which may contribute to the amplification of the carcinogenic effect (diet and passive environmental tobacco exposure (ETS), including seasonal variations in air levels). Only limited data are available on the potency of specific PAHs to induce lung cancer (9) following exposure by inhalation. The most important data correlations provided by the studies performed on animals were focused on demonstration of exposure routes other than inhalation and examined the development of other tumors such as skin carcinomas following dermal contact (13).

The aim of this paper is to assess the levels of carcinogenic and non-carcinogenic PAHs in 31 lung tissues samples from persons with pulmonary cancer and other secondary diagnostics. Secondly, the relationship between concentrations of these pollutants in lung tissue and some clinical parameters in blood serum was also investigated. To the best of our knowledge this is the first study dealing with the contamination status of PAHs in human lung tissue in Romania.

Table 1. Demographic status and pathology of patients

Patients characteristics	
Variables	**n (%)**
Age, *years* (mean±SD)	59.48 ± 10.96
Gender	
Male	28 (87%)
Female	3 (13%)
Non smoking	3 (13%)
Smoking	28 (87%)
Cigarettes/day (mean±SD)	30 ± 9
Primary diagnosis	lung cancer
Secondary diagnosis	
Anemia	20 (65%)
HTN (hypertension)	8 (26%)
Diabetes	3 (9%)

Materials and methods

Study group

A total of 31 subjects from the Clinical Hospital of Pneumology, Iasi, Romania were included in this study. This hospital is a reference centre for the treatment of pulmonary diseases in Moldavia. Lung tissue samples were provided from the anatomical pathology laboratory and were collected for biopsy analysis. Additional clearance was also obtained from the institutional ethics committee for collecting the samples. All subjects were diagnosed with pulmonary cancer confirmed by histological examination. Smoking habits (type and number of cigarettes smoked per day), geographical location of residences, type of domestic heating, and possible exposure through occupational and non-occupational factors were detailed in an exhaustive inquiry form. In addition, information about age, gender and secondary diagnostics was included in the questionnaire (*Table 1*).

None of the subjects reported any occupational or accidental exposure to PAHs sources and through ambient air/food chain contamination, water and soil/dust.

Reagents and materials

All the chemicals and water used were of analytical or HPLC grade. Dichloromethane (DCM), n-hexane (Hex) and acetone for extraction and clean-up were procured from Merck (Darmstadt, Germany). Anhydrous sodium sulfate (for organic trace analysis), supplied by Merck, was purified by heating at 400°C for at least 4h and then kept at 120°C until use. Alumina (80-325 mesh) and silica-gel grade 923(100-200 mesh, 60Å) were manufactured by Sigma Aldrich Inc. (USA). Mix 16 PAHs, naphthalene (Nph), acenaphtene (Ace), acenaphthylene (Acy), fluorene (Fl), phenanthrene (P), anthracene (A), fluoranthene (Flu), pyrene (Py), benzo(a)anthracene (BA), benzo(b)fluoranthrene (BbFlu), benzo(k)fluoranthrene (BkFlu), benzo(a)pyrene (BaPy), dibenzo(a,h)anthracene (DahA), benzo(g,h,i)perylene (BghiP), crysen (Ch), indeno1,2,3-cd-pyren (Ipy) were purchased from Sigma-Aldrich for preparation of external reference standard. All PAHs standards had above 99.99% purity. The PAHs mixture was dissolved in acetonitrile to prepare stock solution; working standard solutions were prepared by mixing stock solutions of each compound at different concentrations in amber volumetric flasks (to avoid light exposure) and stored at 4°C in refrigerator.

Analyses of PAHs

Extraction of PAHs from the lung tissue was carried out according the method reported by Donell et al. (14) with several modifications. Approximately 2 g of homogenized lung tissue sample were mixed with 5 g of Hydromatrix in order to dehydrate and disperse the sample. The ASE extraction was carried out using a Dionex ASE 300 with DCM/acetone (1:1) as extraction solvent at a temperature 100°C and pressure 1500 psi, with two extraction cycles. The extracting solvent was reduced in volume using a rotary evaporator. 3 ml of final extract were prepared for the clean-up stage. Fat content was gravimetrically determined after extraction in DCM.

Clean-up of PAHs

Silica–gel-alumina columns were manually prepared by weighing 3 g of activated silica gel and 3 g of activated alumina into a 10 ml polypropylene SPE cartridge. Elution was carried out using an Alltech SPE manifold, an automatic processing device. The 10 ml of DCM were drained to the top of the column and replaced with 10 ml of hexane. The hexane was drained to the top of the upper sodium sulfate layer and discarded. The sample extract (approximately 3 ml) was loaded on top of the column and PAHs were eluted with 20 ml DCM:Hex (1:1) at a flow rate of 1 ml/min. The eluent was concentrated to approximately 3 ml with a rotary evaporator at 40°C water bath. The final eluate was concentrated under a gentle nitrogen stream at room temperature until near dryness and redissolved in 100 µl acetonitrile.

Instrumentation

All samples were analyzed on a HPLC (ThermoScientific) instrument with fluorescence detector (FLD) using C18 analytical column Thermo Hypersil Green PAH (150mm × 4.6mm i.d., 3.5µm particle size). The elution conditions and detection wavelength program for FLD were described in Romanian Standard for PAHs analysis, 2006 (14) with few modifications. Solvents that constituted the mobile phase were water (A) and acetonitrile (B). The elution conditions were: 0-30 minutes linear gradient 50% B - 100% and then 30-35 minutes 100% B isocratic, and finally, back to the initial conditions for reconditioning of the column. The flow rate was maintained at 1.0 ml/min during whole run of sample as well as standard and the injection volume was 5 µl.

Quantification and quality assurance

Quality assurance samples included spiked matrices, spiked controls, procedure blanks and external calibration standards in acetonitrile. Method limits of quantification (LOQ) for individual PAHs were between 0.002-0.008 ng per gram of wet tissue. The average recoveries calculated by using observed and spike concentrations for PAHs varied from 82% to 95% (RSD <7%) for all PAH compounds. A blank sample was always prepared and run with each set of samples for PAHs analyses during sample preparation.

Biochemical assay

Serum parameters were measured using a Cobas Integra 400 plus (Roche) biochemical auto analyzer. All tests were performed at the Biochemical Laboratory, following standard procedures for clinical biochemistry purposes. The biological markers measured were cholesterol, triglycerides, glucose, calcium, magnesium. C reactive protein was determined using an agglutination method with latex fixation, and fibrinogen was performed using a turbidimetric method.

Hematological assay

White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), Platelet count (PLT), Platelet distribution width (PDW), Platelet large cell ratio (P-LCR), Plateletcrit (PCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean platelet volume (MPV), RBC distribution width-standard deviation (RDW-SD), RBC distribution width-coefficient of variation (RDW-CV), Neutrophil percent (N %), Lymphocyte percent (L%), Monocyte percent (M%), Eosinophil percent (E%), Basophil percent (B%) were assessed using a Sysmex Hematology Analyzer.

Statistical analyses

All statistical analyses were performed using the software package STATISTICA 8 (StatSoft Inc). Nonparametric methods (Mann-Whitney U test and Spearman R correlation coefficient) were used to measure the strength of the association between PAHs concentrations in lung tissue and clinical data. A p-value <0.05 was considered to indicate statistical significance. Normal distribution was tested using the Kolmogorov–Smirnov test.

Results and discussion

The patients selected for this study were diagnosed with lung cancer and other associated diseases such as diabetes (3 patients), HTN (8 patients), and anemia (20 patients). A part of patients included in the study had diabetes and HTN, or diabetes and anemia as secondary diagnoses. Levels of carcinogenic and non carcinogenic pollutants assessed in human lung tissue are presented in *Table 2*.

In all studied cases the sum of non carcinogenic PAHs was higher than for carcinogenic PAHs. Results of PAHs in lung tissue show that patients with pulmonary cancer and HTN as secondary diagnostic have higher concentrations of these pollutants (ΣPAHs non-carcinogenic = 58.02 ng/g wet tissue, ΣPAHs carcinogenic = 10.45 ng/g wet tissue), followed by subjects with diabetes (ΣPAHs non-carcinogenic = 23.10 ng/g wet tissue, ΣPAHs carcinogenic = 3.76 ng/g wet tissue) and anemia (ΣPAHs non-carcinogenic = 32.47 ng/g wet tissue, ΣPAHs carcinogenic = 5.86 ng/g wet tissue) (*Table 2*). Similar study were reported by Goldman in 2001, for 70 cancer-free Caucasian and African-American autopsy donors, but levels of carcinogenic (ΣPAHs = 0.345 ng/g wet tissue) and non-carcinogenic PAHs (ΣPAHs = 0.195 ng/g wet tissue) were lower than in the present study (9).

The most abundant non carcinogenic PAH was Fl with a median concentrations of 14.14 ng/g wet tissue obtained for patients with HTN as secondary diagnoses, amounting for 58% from the total sum of this class.

BaA was the most abundant carcinogenic PAH in patients with HTN (median concentrations of 2.86 ng/g wet tissue) followed by patients with diabetes (1.91 ng/g wet tissue) and anemia (0.55 ng/g wet tissue). BaPy values were higher in case of patients with anemia, with a median concentration of 0.32 ng/g wet tissue. These two carcinogenic pollutants were determined in tobacco smoke (15) and BaA was the most frequent of the all carcinogenic PAHs. The values of BaA in human lung tissue were not a hazard because patients included in study declared a smoking mean level of 30 cigarettes/day (*Table 1*).

A series of recent epidemiologic studies have reported dose-response relationships between the background exposure to POPs (persistent organic pollutants), and various clinical outcomes including type 2 diabetes, metabolic syndrome, or cardiovascular disease (17-19). As shown in *Table 2*, 65% of all cases included in this study have anemia as secondary diagnosis. One of the objectives of this paper is to investigate if levels of PAHs in pulmonary tissue affect clinical parameters of serum and plasma in case of patients with cancer and others diagnoses.

Complete blood count and some serum biochemical parameters and inflammatory markers were tested for each patient before the lung biopsies (*Table 3*). 77% of the all patients showed low values of RBC, HGB and HCT and high values for CRP and fibrinogen. WBC values were high in case of 45% of the patients.

As shown in *Table 3* RBC, HGB and HCT have low values for patients with lung cancer. Severe reductions in these blood elements may lead to anemia, wide interferences with oxygen transport to tissue and may induce hypoxia (20). In case of non carcinogenic PAHs most important correlations were obtained between BghiP and hematological parameters such as HGB ($r = 0.51$, $p = 0.001$), HCT ($r = 0.56$, $p = 0.0003$) and RBC ($r = 0.37$, $p = 0.02$) (*Figure 1*). This could be explained because BghiP has been shown to be responsible for the formation of hemoglobin adducts in mouse blood, and may serve as reliable biomarkers of exposure as well as carcinogenicity (21).

Higher concentrations of BghiP were obtained in case of subjects with lung cancer and anemia as secondary diagnostic with median concentrations of 0.66 ng/g wet tissue. Patients with anemia have also low values of RBC, HGB and HCT. The presence of BghiP in lung tissue could be explained by vehicular traffic (22), if we take into account that 51% of the investigated subjects live in urban areas (*Table 1*).

Table 2. Mean, standard deviation, range (ng/g wet tissue) and positive samples (N) of PAHs for patients with lung cancer and secondary diagnoses such as: diabetes, HTN and anemia

Compound	Diabetes				HTN				Anaemia			
	Mean ± SD	Median	Range	(N)	Mean ± SD	Median	Range	(N)	Mean ± SD	Median	Range	(N)
Non carcinogenic PAHs												
Nph	3.49 ± 1.59	3.63	1.8 - 5.0	3	6.44 ± 12.56	7.00	0 -36.9	8	3.41 ± 3.93	1.83	0 - 16.1	20
Ac	7.6 ± 13.02	0.10	0.09 - 22.6	3	3.27 ± 5.52	13.24	0.1- 15.2	8	4.27 ± 10.1	0.51	0.03 - 45.5	20
Fl	29.19 ± 49.93	0.69	0.03 - 86.8	3	46.38 ± 63.3	14.14	0.17- 162.5	8	6.78 ± 9.38	1.13	0.03 - 35.1	20
P	0.23 ± 0.30	0.08	0.02 - 0.58	3	1.35 ± 1.83	0.04	0.02 - 5.02	8	1.53 ± 1.78	1.03	0.01- 7.5	20
A	2.04 ± 1.66	2.97	0.11 - 3.03	3	2.23 ± 1.94	2.46	0.32 - 5.34	8	4.03 ± 7.51	0.78	0.08 - 30.5	20
Flu	5.46 ± 4.94	5.90	0.31 - 10.2	3	6.45 ± 6.73	6.09	0.38 - 19.51	8	2.43 ± 3.14	1.16	0.05- 10.8	20
Py	4.13 ± 5.06	2.06	0.44 - 9.9	3	5.30 ± 4.73	6.27	0.97- 13.73	8	3.5 5± 3.50	2.20	0.36 - 15.3	20
Ch	5.04 ± 4.11	7.09	0.30 - 7.74	3	8.0 ± 11.2	6.61	0.10 - 35.7	8	5.63 ± 7.65	2.00	0.09 - 25.4	20
BghiP	0.49 ± 0.38	0.58	0.07 - 0.81	3	0.51 ± 0.46	0.16	0.07- 1.49	8	0.80 ± 0.78	0.66	0.04 - 2.4	20
ΣPAHs	57.71 ± 81.03	23.10	3.24 - 177.6		79.96 ± 109	58.02	2.18 - 295.5		32.47 ± 47.8	11.09	0.71 - 189	
Carcinogenic PAHs												
B(b)Flu	0.79 ± 0.74	0.73	0.08 - 1.56	3	1.72 ± 2.35	1.62	0.21-7.14	8	1.19± 1.71	0.39	0.03 -6.91	20
B(k)Flu	0.18 ± 0.08	0.19	0.09 -0.25	3	0.24 ± 0.28	0.03	0.03- 0.90	8	0.57± 0.67	0.34	0.06 - 2.98	20
BaPy	0.10 ± 0.07	0.12	0.02 - 0.17	3	0.29 ± 0.31	0.04	0.04- 0.79	8	0.55± 0.65	0.32	0.05 -2.15	20
DahA	0.53 ± 0.37	0.70	0.11- 0.80	3	1.14 ± 1.43	0.87	0.14- 4.58	8	0.99± 1.1	0.51	0.06 - 4.42	20
BA	1.46 ± 1.16	1.91	0.14 - 2.33	3	3.22 ± 5.35	2.86	0.22- 16.11	8	2.25± 3.1	0.55	0.06 - 12.41	20
Ipy	0.10 ± 0.02	0.11	0.08 - 0.12	3	1.07 ± 1.77	5.02	0.08- 5.02	8	0.29± 0.27	0.21	bdl -1.27	20
ΣPAHs	3.18 ± 2.45	3.76	0.54 - 5.24		7.72 ± 11.5	10.45	0.76 - 34.56		5.86 ± 7.52	2.32	0.26 - 30.1	

Table 3. Biochemical and hematological data in patients with lung cancer

Parameters	Units	Mean	SD	Min	Max	Reference range
Total cholesterol	(mg/dL)	207	24	174	276	100-200
Triglycerides	(mg/dL)	153.35	37.47	102.00	255.00	< 200
Glucose	(mg/dL)	117	32	74	220	60-110
Calcium	(mg/dL)	8.85	0.48	8.10	10.40	8.8-10.8
Magnesium	(mg/dL)	2.13	0.22	1.70	2.60	1.7-2.5
Fibrinogen	(g/L)	4.62	1.72	2.24	9.50	2-4
C-reactive protein	(mg/L)	16.4	12.6	0	48	0
WBC	$10^3/\mu L$	9.8	2.9	4.3	14.8	4-10
RBC	$10^6/\mu L$	4.02	0.6	2.8	5.3	4.25-5.5
HGB	g/dL	11.85	2.11	8.00	16.50	12-16
HCT	%	35.63	5.59	24.70	47.60	42-52
MCV	fL	81.61	20.49	28.70	103.60	37-47
MCH	pg	31.53	10.58	22.00	88.40	28-33
MCHC	g/dL	38.11	28.03	30.10	194.00	28-35
PLT	$10^3/\mu L$	346.41	153.09	125.00	735.00	150-450
RDW-SD	fL	45.41	6.76	37.90	66.10	37-54
RDW-CV	%	14.48	2.66	12.30	25.40	11-16
PDW	fL	13.72	1.99	10.10	19.30	9-15
MPV	fL	10.82	0.97	9.10	13.10	9-13
P-LCR	%	32.07	7.42	18.10	48.90	13-43
PCT	%	0.35	0.12	0.16	0.64	0.17-35
Neu	%	68.40	10.69	48.80	89.40	50 - 70
Ly	%	19.36	8.64	3.80	39.40	20 - 45
Mo	%	9.23	2.09	2.60	12.80	4 - 10
Eo	%	2.65	2.70	0.10	11.50	1 - 4
Ba	%	0.37	0.27	0.00	1.30	0 - 1

Significant positive correlations were obtained between carcinogenic PAHs such as BaPy and PLT ($r = 0.44$, $p = 0.01$), BaPy and PCT ($r = 0.42$, $p = 0.01$). However, the suitability of BaPy as an indicator of carcinogenic PAH has been questioned by findings on the presence of more potent PAHs such as dibenzo(a,h)anthracene (DahA) and dibenzo(a,l)pyrene (8). Correlations between DahA and HCT ($r = 0.43$, $p = 0.01$) and PCT ($r = 0.38$, $p = 0.03$) were observed. Studies on PAHs show that BaPy may be relevant in cases where the specific populations are chronically exposed to BaPy emanating from hazardous waste sites, where chronic intake occurs via food or tobacco, or during occupational exposure (22). In a study on BaPy in tobacco smoke were reported concentrations of 20-40 ng/cigarette (23). Studies on the rats show that, of the blood parameters tested, only the red cell-associated parameters appeared to be affected by subchronic dos-

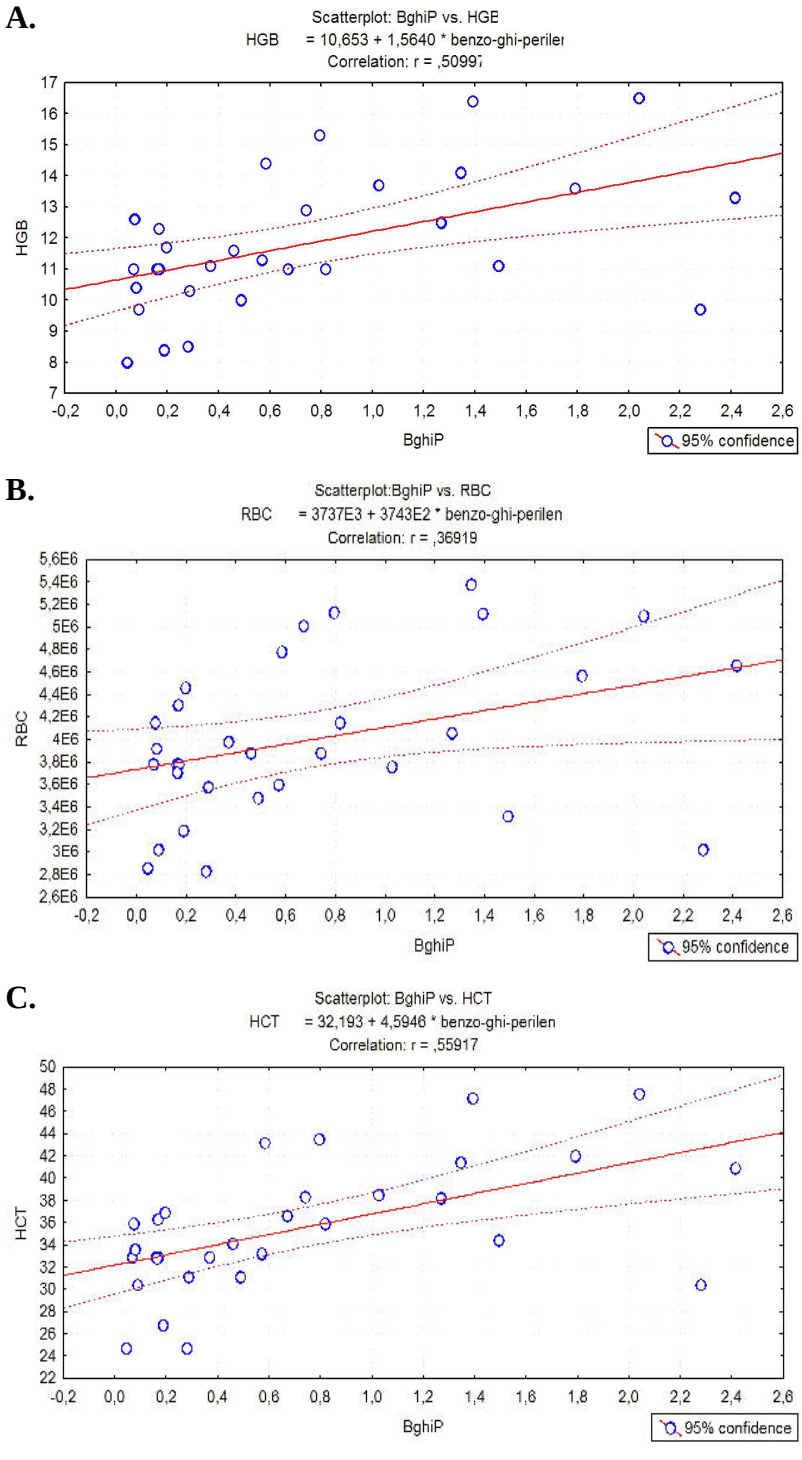

Figure 1. Linear regression between blood HGB (a), RBC (b), HCT (c) and BghiP in patients with lung cancer.

ing with BaPy while HCT was depressed only at a higher dose for this carcinogenic pollutant (24).

Results of Mann-Whitney U test show significant differences between patients with HTN and anemia for B(k)Flu. This carcinogenic PAH was found in higher concentrations in case of subjects with anemia, with a median concentration of 0.34 ng/g wet tissue and has also been demonstrated to be a tumor initiator in mice (25).

Statistical tests applied in case of serum biochemical parameters and concentrations of PAHs in lung tissue show positive correlations for BaA with CRP ($r = 0.39$, $p = 0.02$) and fibrinogen ($r = 0.36$, $p = 0.04$). Fl, the most abundant non carcinogenic PAH, was correlated only with inflammatory markers, CRP ($r = 0.38$, $p = 0.03$) and fibrinogen ($r = 0.50$, $p = 0.004$). Cigarette smoke could represent a more important source of Fl (15) in lung tissue but we cannot exclude sources as biomass combustion and wood burning (22) because 49% of the total investigated subjects live in rural areas.

There are several limitations to this study. No classification could be made on exposure of PAHs for smokers and non-smokers because the higher number of the first donor group (28, versus 3 for non-smokers). As shown in *Table 1*, among the 31 subjects enrolled, 28 declared to be active smokers with a mean level of 30 cigarettes per day. This represents a true risk for human health because it was estim-

ated by the FTC machine method that a smoker is exposed to about 1-30 g PAHs/ day/ pack of cigarettes (3). Also, because only 10% of the total samples belong to women and 90% for men no comparison could be made between the results obtained for these two categories.

Conclusions

Patients with cancer and HTN as secondary diagnosis have higher lung concentrations of PAHs, followed by the patients with diabetes and anemia. Mean levels of non carcinogenic PAHs were higher than carcinogenic PAHs in all three cases studied. The majority of subjects have decreased values for blood RBC, HGB, HCT and higher values of CRP and fibrinogen. Such results might point out that presence of the PAHs in lung tissue cancer may affect hematopoietic system and inflammation markers. Additional studies are needed to confirm the alteration of serum biochemical and blood hematological parameters due to the presence of PAHs in humans.

Acknowledgements. The authors are grateful to Fiterman Pharma for funding support in the infrastructure for PAHs determination.

References

1. Singh VK, Patel DK, Jyoti SR, Mathur N, Siddiqui MKJ. Blood levels of polycyclic aromatic hydrocarbons in children and their association with oxidative stress indices: An Indian perspective. Clin Biochem, 2008; 41:152-161
2. Kotin P and Falk HL, Polluted urban air and related environmental factors in the pathogenesis of pulmonary cancer, Chest. 1964; 45: 236-246.
3. Hoffmann D and Hoffmann I. The changing cigarette, 1950–1995. J. Toxicol Environ Health. 1997; 50: 307–364.
4. Klánová J, Čupr P, Holoubek I, Application of passive sampler for monitoring of POPs in ambient air. Part II: Pilot study for development of the monitoring network in the Central and Eastern Europe (MONET_CEEC), 2006. Masaryk University Brno ISBN 978-80-210- 4393-0.
5. Stockholm Convention on Persistent Organic Pollutants (POPs) (http://chm.pops.int/Countries/NationalImplementation/tabid/253/language/en-US/Default.aspx http://chm.pops.int/Convention/POPsReviewCommittee/tabid/60/language/en-US/Default.aspx.
6. IARC (1986), Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1986; 38. Appendix 2, pp. 389–394. Lyon, France.
7. US EPA, Environmental Protection Agency, Carcinogen Assessment of Coke Oven Emissions. Final report. Office of Health and Environmental Assessment. Washington, DC. 1984; EPA 600/6-82-003F. NTIS PB 84-170181.
8. Okona-Mensah KB, Battershill J, Boobis A, Fielder R, An approach to investigating the importance of high potency polycyclic aromatic hydrocarbons (PAHs) in the induction of lung cancer by air pollution, Food Chem Toxicol. 2005; 43:1103–1116.
9. Goldman R, Enewold L, Pellizzari E, Beach JB, Bowman ED, Krishnan SS et al, Smoking Increases Carcinogenic Polycyclic Aromatic Hydrocarbons in Human Lung Tissue, Cancer Res. 2001; 61: 6367-6371.
10. Doll R and Peto R, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. 1981; 66: 1191–1308.
11. Conney AH, Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. Cancer Res. 1982; 42: 4875–4917.
12. Dipple A. DNA adducts of chemical carcinogens. Carcinogenesis (Lond.). 1995, 16: 437–441.
13. WHO, Environmental Health Criteria 202: Selected non-heterocyclic polycyclic aromatic hydrocarbons. IPCS, International Programme on Chemical Safety. World Health Organisation, 1998, Geneva. On line at: http://www.who.int/cancer/en/index.htm.
14. Donell SF, Wang B, Ramirez JA, McDonald SJ, Price R, Brooks JM, Extraction of biological tissues for aromatic and chlorinated hydrocarbons. In: Kimbrough, KL., G.G., Lauenstein and Johnson W. E., (Eds). Organic Contaminant Analytical Methods of theNational Status and Trends Program: Update 2000-2006. NOAA Technical Memorandum NOS NCCOS 2006; 30, 9-15.
15. Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami D, Hecht SS. Analysis of 23 Polycyclic Aromatic Hydrocarbons in Smokeless Tobacco by Gas Chromatography-Mass Spectrometry, Chem. Res. Toxicol. 2010. 23:66–73.
16. SR EN ISO 17993/2006. Water quality. Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction.
17. Ha MH, Lee DH and Jacobs DR, Jr. Association between Serum Concentrations of Persistent Organic Pollutants and Self-Reported Cardiovascular Disease Prevalence: Results from the National Health and Nutrition Examination Survey 1999–2002, Environ Health Perspect. 2007. 115(8): 1204-1209.

18. Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002, Diabetes Care. 2007a. 30: 622–628.
19. Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002, Diabetologia. 2007b. 50: 1841–1851.
20. Knuckles ME, Inyang F, Ramesh A. Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. Toxicol Sci. 2001; 61: 382-388.
21. Hurst HE. and Myers, RS. Biomarkers for Air Pollutants: Development of Hemoglobin Adduct Methodology for Assessment of Exposure to Butadienes and Polycyclic Aromatic Hydrocarbons, 2004. SEER project of SIP:20 Experimental Program To Stimulate Competitive Research (EPSCoR) From The Commonwealth Of Kentucky (2001-2005). EPA Grant Number: R829419. University of Louisville.
22. Agarwal T, Khillare PS, Shridhar V, Ray S, Pattern, sources and toxic potential of PAHs in the agricultural soils of Delhi, India, J Hazard Mater. 2009; 163: 1033–1039.
23. Lioy PJ. Human exposure assessment: A graduate level course. J Expo Anal Environ Epidemiol. 1991; 1: 271-281.
24. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999; 91: 1194-1210.
25. ATSDR. Agency for Toxic Substances and Disease Registry. Toxicological profile for polycyclic aromatic hydrocarbons, 1995, Atlanta. http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf