Original Research

Measurements of Chemical Elements in Occupationally Exposed Hair and Their Correlation with Nephrotoxicity Markers in Urine

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Abstract

The possibility of early detection of subclinical changes in the organism of individuals occupationally exposed to chronic action of chemical toxins is a continual subject of study.

The analysis of metals in hair is a permanent record of length of exposure and metal circulation. However, such analysis alone cannot be used for the diagnosis of disease. In the presented work we determined the concentrations of Pb, As, Ni, and V in the hair and neutrophil lipocalin (NGAL) in urine, and conducted the correlation between these chemical elements and specific nephrotoxicity indicators in urine: NGAL, N-acetyl- β-D-glucosaminidase (NAG, its isoform NAG-B), β-galactosidase (β-GAL), β-glucuronidase (β-Gr), alanylaminopeptidase (AAP), glutathione transferases (α and π GST), β_2-microglobulin (β_2M), retinol binding protein (RBP), sialic acids (SIA), albumins and total protein. The studies were conducted on 29 employees of the Polish Fuel Company and on 19 healthy individuals without occupational exposure to chemicals.

Keywords: occupational exposure, metals, hair, gasoline, lipocalin

Introduction

Chronic occupational exposure is more and more often the reason for widespread health issues. Early detection of risk permits time for adequate measures to avoid potentially unfavorable effects. A solitary examination of blood and urine is often not sufficient for the detection of chronic exposure (at a low level). The analysis of human hair could be a biological marker of occupational and environmental exposure. It is an indicator of metabolic processes occurring in cells. The concentrations of some elements in the hair are significantly higher than in the blood and other tissues. Analysis of toxic metals in hair is a stable record of the time of exposure and circulation of these metals [1, 2]. In the present work the correlation between the chemical elements in the hair and specific urine markers of nephrotoxicity is measured. Such studies seem to be useful in the early evaluation of the results of chronic occupational exposure in employees of the Fuel Company, since fuels are a source of nephrotoxic metals [3].

Contact with gasoline is an important problem in contemporary toxicology. The danger of toxic exposure is a result of contact with liquid or volatile phase, most often during production, transportation, storage, distribution and utilization. Although the air in refineries and gas stations contains low concentrations of volatile hydrocarbons, small, repeated leakage of gasoline concentrations that transiently exceed the norms lead to toxic exposure. Toxicity of

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Table 1. Average concentrations of studied chemical elements in subjects occupationally exposed and in control group.

		age [years]	V [ppb]	Ni [ppb]	As [ppb]	Pb [ppb]
Examined Group	$\bar{X}$	46.76	101.45	1,393.81*	348.38*	5,996.19*
	OS	6.85	66.28	761.98	209.68	4,621.62
	n	29	29	29	29	29
Comparison Group	$\bar{X}$	47.26	118.02	655.31	203.3	2,190.65
	OS	4.48	53.31	446.08	60.71	1,379.76
	n	19	19	19	19	19

*Statistically significant values.

many metals is connected not only with the properties of their organic derivatives used as antiknock agents, but most often with inorganic compounds exhausted in combustion gases spread in the environment and accumulating in the human organism [4, 5].

A permanent exposure to microdoses of the toxins together with their high assimilation and accumulation in parenchymatous organs usually leads to subclinical disorders of vital functions of the organism. Chronic exposure even to small concentrations causes accumulation of microdamages and obscure pathologic processes, which may appear as clinical symptoms after many years [6].

The objective of our work was the determination of certain chemical element concentrations in the hair and NGAL in the urine, as well as the examination of the correlation of these chemical elements with specific nephrotoxicity indicators, determined earlier, in employees exposed gasoline vapors [7, 8].

Materials and Methods

The urine and hair of 29 workers of the Polish Fuel Company employed directly in the fuel distribution department were used in the studies. The cohort consisted of men aged 34 to 63 years (average 46.76 years) who were employed from 6 to 32 years (average 16.33 years). These workers were exposed to gasoline compounds: alkanes, alkenes, ethers and heavy metals. The control group consisted of 19 healthy individuals (average age 47.26 years) from the countryside without occupational exposure to chemicals.

The first morning urine, from workers in a fasting state, was collected into polyethylene containers without conservatives. Morphotic elements were removed by centrifuge at 3,000 rpm for 10 minutes. The urine was diluted to eliminate inhibitors and activators. Material was stored at -80ºC until analyzed.

Analytical hair determinations were carried out in a laboratory certified by the Polish Centre for Accreditation (AB 696), which is a member of ILAC-MRA. Hair (directly after washing with shampoo and drying) was sampled from the nape of the neck. Samples were washed using acetone and distilled water [9]. Hair samples were digested in a microwave digestion system (MILESTONE, Italy) in closed Teflon bombs. Hair (ca. 1g) was mineralized with concentrated nitric acid (5 ml). The reagent and digestion conditions were chosen in order to achieve complete mineralization and decomposition of solid phase into liquid phase. All reagents used were of supra pur grade (from Merck Company). After digestion, the solutions were filled up to 50 ml with deionized water.

The concentrations of Pb, As, Ni, and V were determined. Multielemental analysis of digested hair samples was performed with inductively coupled plasma-mass spectrometry with mass detection (ICP-MS) controlled by computer cooperating with analytical system UltraMass 700 (VARIAN, Australia).

The operating parameters of the ICP-MS: Plasma flow – 16 l/min, Auxiliary flow – 1 l/min, Sampling depth – 5.8 mm, Radio frequency power – 13 W, Pump rate -20 rpm, Number of replicates – 3.

The samples were analyzed in three repeats (the relative standard deviation of the measurement was lower than 3%).

Analytical process was controlled by the use of matrix standard NCS Reference Material – Human Hair NCS ZC8 1002 from China National Analysis Center [10].

Activities of NAG, NAG-B, β-GAL, β-Gr in the urine were determined colorimetrically [7].

GST α and π isoenzymes, NGAL and concentrations of β_2M and RBP were determined immunoenzymatically using the ELISA method (Biotron, Bioporto, R&D Systems).

Total protein, free SIA, albumins were determined using the turbidimetric method (Bering TurbiTimeSystem) [8].

Statistical analysis was performed by t-Student test or U Mann-Whitney test using the Statistica 7.1 statistical package. Correlation between parameters was analyzed using the Spearman or Pearson linear correlation coefficient. In all analyses the coefficient $p < 0.05$ was regarded as statistically significant.

Table 2. Average concentrations of studied chemical elements depending on the length of employment in subjects occupationally exposed.

Employment duration		V [ppb]	Ni [ppb]	As [ppb]	Pb [ppb]
Over 18 years	$\overline{X}$	113.22	1,730.78	358.56	8,794.83*
	OS	66.45	766.58	194.04	5,578.98
	n	10	10	10	10
Below 18 years	$\overline{X}$	95.26	1,216.46	343.03	4,523.22
	OS	67.14	716.28	222.44	3,319.91
	n	19	19	19	19

* Statistically significant values

Results and Discussion

In the presented work the concentrations of Pb, As, Ni and V in the hair and the concentration of neutrophil lipocalin (NGAL) in the urine were determined. We estimated a correlation between the concentration of these metals and specific nephrotoxicity indicators in urine such as NGAL, lysosomal enzymes – NAG, its isoform NAG-B, β-GAL, β-Gr, brush border enzymes – AAP, glutathione transferases (α and π GST – specific for proximal and distal tubules of the kidney), and parameters of glomerular filtration process – sialic acids , total protein, albumins, low-weight proteins: β_2M, RBP [7, 8].

In the studied group exposed to gasoline vapors, a significantly higher concentration of Pb, Ni and As in hair was observed as compared to the control group ($p<0.0001$). Concentrations of Ni (1,393.81 ppb) and Pb (5,996.19 ppb) exceeded the results in the control group by two times (Table 1). Our results are in the agreement with those obtained by Vishwanathan et al. [11] in India, who observed elevated concentrations of Pb and Ni in the hair of car drivers exposed to combustion gas inhalation. Increased concentrations of Pb in hair and serum have been observed in children from copper mining regions and car mechanics [12, 13].

In the studied subjects, only the concentration of vanadium in the hair was similar to the control group (Table 1). Similar results were obtained by Strzelczyk et al. [1] in studies conducted on workers of an oil plant in Płock.

Extremely low concentrations of vanadium in the hair in comparison with other analyzed chemical elements may result from its specific chemical character. Vanadium belongs to metals loosely connected to the proteins of hair. Moreover, international studies have reported that concentrations of vanadium in hair in the Polish population is the lowest among the studied populations (the highest average concentration of this element was noted in the USA) [14, 15].

Our studies revealed that in 93% of the fuel workers the concentration of As in hair was elevated.

The hair and nails are recognized indicators of environmental and occupational exposure to As. They may absorb arsenic compounds with 89% effectiveness for As^{III} and 86% for As^{V}. Mandal et al. [16] demonstrated 20 times higher concentration of this element both in the hair and nails of studied subjects. Arsenic incorporated into keratin of hair may serve as a long-term exposure indicator [17].

Kakkar et al. [2] observed certain limitations of the blood as a biomarker of exposure to As. It results from the difficulty in distinguishing between the level of exposure to inorganic As delivered with drinking water and organic As delivered to the organism with food. Arsenic is quickly metabolized and excreted in the urine. Therefore, the sum of metabolites of this element in the urine treated as an indicator of the short exposure, without considering diet intake, may lead to erroneous interpretations [18].

In our studies, during analysis of hair chemical element concentrations, we also took into consideration the length of employment in harmful conditions and the age of the workers.

In workers with longer time of work (above 18 years) the average concentration of the chemical elements in the hair was higher than in the hairs of those who had worked fewer years (Table 2). A statistically significant difference ($p<0.01$) was observed only in the case of Pb.

Average concentration of Pb in workers working longer than 18 years was 8,794.83 ppb and in workers working shorter 4,523.22 ppb. While increasing age, the larger amount of the metals, especially lead, is being revealed from the bones to circulation. The lead influx from the bones is an important source of circulating lead. That is why the concentration of this metal is increasing in organs and hair [19].

Higher concentrations of metals in the hair of workers who worked longer are concordant with studies of Afridi et al. [20]. In their studies they observed positive correlation between concentration of Pb in the hair, urine and blood, and the period of employment.

The presented studies confirmed the choice of hair as diagnostic material for the estimation of occupational exposure of the workers of the oil company.

The analysis of differences in average concentrations of chemical elements in the studied and control group suggests that the higher concentration of the chemical elements

Table 3. Correlation between chemical elements analyzed in the hair and biochemical indicators in the urine of occupationally exposed workers.

		V	Ni	As	Pb
NGAL		r = -0.3019	r = **0.5007** *	r = **0.5964***	r = 0.3353
		p =0. 1115	p = **0.0057**	p = **0.0006**	p = 0.0753
NAG		r = -0.0434	r = **0.6090** *	r = **0.5341***	r = **0.7384***
		p = 0.8231	p =**0.0005**	p = **0.0028**	p = **0.0000**
NAG-B		r = -0.0526	r = **0.3822** *	r = **0.3946***	r = **0.6681***
		p = 0.7864	p = **0.0407**	p = **0.0342**	p = **0.0001**
β-GAL		r = 0.1491	r = -0.2742	r = -0.0705	r = 0.0212
		p = 0.4401	p = 0.1500	p = 0.7165	p = 0.9131
β-Gr		r = 0.1372	r = -0.1337	r = -0.1364	r = 0.1600
		p =0.4779	p = 0.4894	p = 0.4806	p = 0.4069
AAP		r = 0.1319	r = 0.0999	r = -0.1726	r = 0.1859
		p =0.4950	p = 0.6062	p = 0.3705	p = 0.3342
α-GST		r = -0.0606	r = **0.3788** *	r = 0.2006	r = 0.3328
		p = 0.7550	p = **0.0427**	p = 0.2967	p = 0.0777
π-GST		r = -0.1081	r = 0.3570	r = 0.2586	r = 0.2188
		p = 0.5768	p = 0.0573	p = 0.1756	p = 0.2542
$β_2M$		r = 0.3125	r = 0.3256	r = 0.1310	r = 0.2493
		p = 0.0606	p = 0.0848	p = 0.4981	p = 0.1922
RBP		r = 0.2256	r = 0.3701	r = 0.1974	r = 0.2701
		p = 0.2890	p = 0.0751	p = 0.3551	p = 0.2019
SIA		r = 0.0407	r = -0.1110	r = 0.2269	r = -0.1746
		p = 0.8340	p = 0.5666	p = 0.2366	p = 0.3650
Albumins		r = 0.2697	r = 0.0063	r = 0.0803	r = 0.1150
		p = 0.1572	p =0.9742	p = 0.6789	p = 0.5525
Total protein		r = 0.2540	r = 0.3000	r = 0.0641	r = 0.2805
		p = 0.1836	p = 0.1138	p = 0.7412	p = 0.1405

* Statistically significant values $p < 0.05$.

in the hair in the exposed group may be accompanied by subclinical renal damage, which was confirmed by the increased activities of several sensitive indicators of nephrotoxicity in the urine of these workers [7, 8]. Numerous scientific centers in the world conduct studies on the concentrations of various elements in hair. However, there is a lack of studies which take into consideration concentration of chemical elements in the hair of the group exposed to certain xenobiotics in connection with early indicators of the damage of certain organs such as kidneys, for example.

By means of correlation analysis we tried to estimate the connection between chemical element content in the hair and specific indicators of nephrotoxicity in urine.

Table 3 contains correlation coefficients (r) and relevant coefficients of significance of the difference (p), which describe correlation of concentration between hair chemical element concentrations and the excretion of studied biochemical indicators in urine.

We demonstrated a strict connection between concentration of Pb, As and Ni in hair and the activity of NAG and NAG-B in urine. It may be suggested that along with increasing concentration of these chemical elements in hairs the risk of interstitial damage of the kidney rises, which is expressed by the increase in NAG and NAG-B activities [21]. The strongest correlation between chemical element concentrations in the hair and biochemical indicators in urine was observed between Pb and activities of

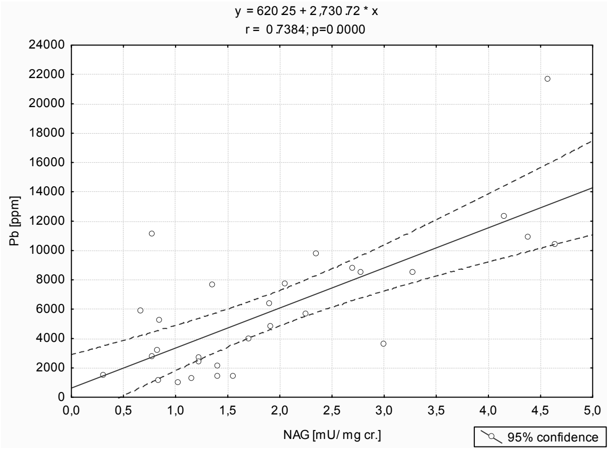

Fig. 1. Dependence of NAG activity in urine on the content of Pb in the hair of workers exposed to gasoline vapor.

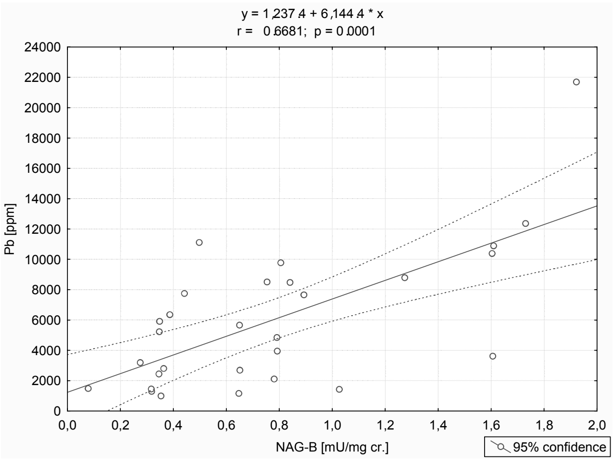

Fig. 2. Dependence of NAG-B activity in urine on the content of Pb in the hair of workers exposed to gasoline vapor.

NAG and NAG-B; the correlation was statistically significant ($p<0.0001$) (Figs. 1, 2).

But significant correlation between blood level of Pb and secreted NAG in urine was demonstrated by Mortada et al. [4], who studied the influence of exposure to Pb from car combustion gases on the kidneys' function in traffic police.

Similarly as in our studies, the authors did not find any significant correlation between the concentration of low-weight protein β_2M and the concentration of Pb in the studied biological material.

In the presented study, using correlation analysis, we tried to estimate the usefulness of NGAL protein, a newly discovered biomarker of renal damage. Although the function of human neutrophil lipocalin NGAL is not fully recognized, it is known that a concentration of this protein increases after hypoperfusive-hypoxemic damage of the kidney canaliculi [22].

Although the average activity of NGAL in the studied group (0.46 ug/l) was close to the control group (0.42ug/l), in 41% of studied subjects the value of this indicator was elevated. The elevation of NGAL concentration in the studied group was significantly ($p<0.005$) correlated with an increase in arsenic ($r= 0.5964$, $y = 131.68 + 511.75 \cdot x$) and nickel ($r=0.5007$, $y = 732.71 + 1561.22 \cdot x$) concentrations in the hair. The conducted studies may suggest that NGAL may serve as a prognostic marker of Ni and As nephrotoxicity, which was confirmed by significant correlations with these chemical element concentrations in the hair of examined individuals.

Additionally, our studies demonstrated the correlation between Ni concentration and α-GST activity ($r = 0.3788$, $y = 1245.33 + 55.99 \cdot x$). α-GST isoenzyme belongs to glutathione transferase, selectively localized in proximal canaliculus, which permits the localization of nephron damage in the examined workers [23].

It can be assumed that along with the increase of Ni concentration in the hairs of studied subjects, the damage of cells of the epithelium of proximal canaliculus increases, which was reflected by the elevation of α-GST activity.

Conclusions

1. Significantly higher concentrations of As, Ni and Pb in the hair of workers of the Polish Oil Company were observed. The hair can serve as additional diagnostic material for occupational exposure.
2. Significant correlations of chemical element concentrations with analyzed biochemical indicators indicate the usefulness of hair as a diagnostic material in the estimation of exposure.
3. A significant connection between the length of employment and concentration of chemical elements in the hair of the examined workers was observed.

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