Repeated study of lead impact biomarkers in occupationally exposed workers

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The activity of Δ-aminolevulinic acid dehydratase (Δ-ALAD) and excreted with urine Δ-aminolevulinic acid (Δ-ALA) concentration is one of the most sensitive biomarkers of lead impact. Toxic lead effects decrease the activity of Δ-ALAD in the blood, while the level of Δ-ALA in urine increases. The aim of this study was to evaluate the impact of lead on occupationally lead-exposed workers (n = 13) from Joint-Stock Company "Lietuvos energija" Kaunas Branch "Kauno Elektros Tinklai". The obtained data were compared to data of the control group (n = 19).

The content of lead and Δ-ALAD activity in blood were measured in 1998 and 2000 and the concentration of lead and Δ-ALA in urine in 1999 and 2000. Lead concentration was determined with a Perkin–Elmer/Zeeman 3030 ETG atomic absorption spectrophotometer. The Δ-ALAD activity in blood and Δ-ALA concentration in urine were measured with a spectrophotometer. The t test for dependent and independent values for statistical analysis of data was applied.

Δ-ALAD activity in the worker's blood in 2000 was found to be significantly lower than shown by the same workers in previous screening in 1998 and by the control group (respectively, $p < 0.001$ and $p < 0.001$). The blood and urine lead level of workers both in 1998 and 2000 was higher as compared to control group (respectively, $p < 0.01$ and $p < 0.01$). Although the established lead concentration in occupationally lead-exposed workers did not exceed the permissible level, a significantly decreased blood Δ-ALAD activity and increased urine Δ-ALA level *versus* were observed the control group. Δ-ALAD activity is one of the most sensitive biomarkers of the impact of low lead doses.

Key words: lead impact biomarkers, Δ-aminolevulinic acid dehydratase (Δ-ALAD), Δ-aminolevulinic acid (Δ-ALA), occupational exposure to lead

INTRODUCTION

Lead is one of the most ubiquitous toxic metals. In industry, inorganic lead oxides are used (various lead salts are present in ceramic and tile glaze, oil paints and enamel, paper printing ink (presently out of use), galvanic elements, as components of some medicines and cosmetics, etc.,) as well as organic lead compounds (tetraethyllead in fuel is not used any more).

Lead enters the human body with contaminated food, drinking water, polluted air or via occupational exposure to lead. Alkylated organic lead compounds can enter the body even through healthy skin. Exposure to lead causes chronic or acute intoxication. The extent of lead impact depends on lead exposure, dose and the degree of effects it causes in the body. Exposure to lead causes haematological, neurobehavioural and cardiovascular disorders, provokes renal deficiency and damages the reproductive system.

Lead damages the haematopoietic system and haeme biosynthesis. Lead inactivates enzymes by binding to mercapto, amino and carboxyl groups of enzymes. Lead inhibits the activity of one of haeme biosynthesis enzyme – Δ-aminolevulinic acid dehydratase (Δ-ALAD), and hence the concentration of Δ-aminolevulinic acid (Δ-ALA) in urine increases. There is a correlation between blood lead level and Δ-ALAD activity in blood (Chalevelakis et al., 1995, Morita et al., 1993; Rothe et al., 1980; Takebayashi et al., 1993). The activity of Δ-ALAD in blood is one of acceptable lead impact biomarkers (Polizopoulou et al., 1994; Strom et al., 2002). Significantly higher Δ-ALA urine levels have been established for subjects occupationally exposed to lead

(Hisgashikawa et al., 2000; Molina-Ballesteros, 1976), while even low lead levels can cause a decrease of Δ-ALA concentration in urine (Makino et al., 2000). Therefore, in case of low blood lead level, Δ-ALAD activity is a more sensitive lead impact biomarker than Δ-ALA concentration changes in urine.

The objective of this repeated study was to evaluate changes of lead impact biomarkers – Δ-ALAD activity in blood and Δ-ALA concentration in urine for workers occupationally exposed to lead.

MATERIALS AND METHODS

In the repeated study there were involved workers occupationally exposed to lead (electricians, cable fitters, electro-adjusters, and cutout renovators) from Joint-Stock Company "Lietuvos Energija" Kaunas Branch "Kauno Elektros Tinklai". Due to its specific character only men perform this job and therefore only mail subjects (n = 13) were investigated. The workers are exposed to lead and solder vapour indoors. The obtained data were compared to the data obtained from a control group of men who have never been occupationally exposed to lead (n =19). The content of lead and Δ-ALAD activity in vein blood were measured in 1998 and 2000, while the concentration of lead and Δ-ALA was checked in spot urine in 1999 and 2000.

The lead concentration in blood and urine was determined with a electrothermal graphite furnace atomic absorption spectrophotometer Perkin-Elmer/Zeeman 3030. The vein blood was obtained by single syringes and using heparin "Biochemie" (Biochemie GmbH, Vienna, Austria) as an anticoagulant. Spot urine specimens were collected into plastic tubes. The modified analysis method (Schlemmer, 1989) for heavy metal concentration detection in biological samples was applied. There were used contamination-free, repeated 2.4 M nitric acid washed and followed by repeated deionized pure water rinse minisorption plastic tubes and labware for all media. Therefore, analyses were controlled by inclusion of internal quality control materials.

Spectrophotometry for measuring Δ-ALAD activity in blood and Δ-ALA content in urine was applied. The exact quantity of porphobilinogen is synthesised under the standard conditions with Δ-aminolevulinic acid added. The colour intensity of porphobilinogen and p-dimethylaminobenzaldehyde compound is proportional to porphobilinogen concentration. Thus, the Δ-ALAD activity was estimated according to porphobilinogen concentration as described in (Berlin et al., 1974; Архипова и др., 1988). The Δ-ALA concentration in urine was determined according to a modified methodology (Rijks, 1974; Семенова, 1985). The urine was purified by absorbent carbon, later on adding the 4-dimethylaminobenzaldehyde solution and measured with a spectrophotometer. Also, the levels of creatinine in urine were determined according to the Jaffe methodology (Jaffe, 1986).

The workers were requested to answer a questionnaire about their smoking and alcohol consumption habits. Those who smoked more than one cigarette per day were considered as smokers, while as drinkers were treated those who consumed heavy liquors (vodka, cognac, liqueur, kinds of brandy) once per month or more frequently.

The t test for dependent and independent values of statistical data of analysis was applied (Daniel, 1995).

RESULTS AND DISCUSSION

The mean (min–max) age of workers occupationally exposed to lead was 42.11 (range, 21–67) and of the control group 47.89 years (range, 29–66). The mean (min–max) service length for workers was 15 years (1–52). The established activity of blood Δ-ALAD in 1998 was statistically significantly higher than in the same workers in 2000 (0.595 μmol/ls and 0.403 μmol/ls, respectively, $p < 0.001$). The obtained data of lead concentration and lead impact biomarkers for occupationally exposed workers are presented in Table.

Table. **Activity of Δ-ALAD in blood, blood lead concentration and levels of lead and Δ-ALA in urine of workers occupationally exposed to lead, 1998–2000**

Indices	Year 1998	Year 1999	Year 2000
	(mean, 95% CI)	(mean, 95% CI)	(mean, 95% CI)
Δ-ALAD in blood (μmol/ls)	0.595* (0.52–0.67)	–	0.403 (0.33–0.48)
Pb in blood (μg/dl)	7.12 (2.76–11.48)	–	5.39 (3.69–7.08)
Δ-ALA in urine (μmol/g creatinine)	–	12.82 (8.93–16.71)	10.51 (7.37–13.65)
Pb in urine (μg/l)	–	4.20* (2.12–6.28)	8.08 (3.85–12.30)

* $p < 0.001$.

The blood lead concentration was lower in 2000 μmol/ls than in 1998 and did not differ significantly (5.39 μg/dl and 7.12 μg/dl, respectively, $p > 0.05$). The mean concentration of Δ-ALA in urine in 2000 decreased to 10.51 μmol/g · creatinine against 12.82 μmol/g · · creatinine in 1999. Therefore no significant differences were established. The mean lead concentration in urine in 1999 was significantly lower than in 2000 (4.20 μg/l and 8.08 μg/l, $p < 0.001$). These figures probably show the level of lead excretion from the body.

We also evaluated the lead impact biomarkers in respect of smoking as one of the factors that together with occupational lead exposure could have a serious impact on the human body. Almost half of workers were smokers. The blood lead level was found to be higher in smoking than in non-smoking workers (Fig. 1). No significant differences were observed.

There was a tendency of blood lead level decrease. Moreover, a decrease was observed in blood Δ-ALAD activity of smoking and non-smoking workers. Therefore the Δ-ALAD activity level in the blood of smoking workers *vs*. non-smoking workers remained lower. These data are presented in Fig. 2. However, our study showed no effect of alcohol consumption on changes in lead impact biomarkers.

In terms of the evaluation of the impact of occupational exposure on changes in lead biomarkers the obtained data were compared to those of the control group. The established mean (95% CI) of Δ-ALAD activity in the control group was 0.66 μmol/ls (0.60–0.73), blood lead level – 2.66 μg/dl (1.86–3.47), Δ-ALA content in urine – 8.88 μmol/g · creatinine (6.98–10.78), and urine lead level was 1.95 μg/l (1.36–2.55). In 2000 a significant decrease of Δ-ALAD activity was established in workers' blood *vs*. control group ($p < 0.001$), while in 1998 no significant difference was observed ($p > 0.05$). Therefore blood lead concentration in workers *vs*. control group in 1998 and 2000 was significantly higher, respectively $p < 0.01$ and $p < 0.01$. Also, lead in urine of workers *vs*. control group in 1999 and 2000 was significantly higher, $p < 0.05$ and $p < 0.01$, respectively. The content of Δ-ALA in the urine of workers *vs*. control group was significantly higher in 1999 ($p < 0.05$), while in 2000 no significant differences were detected ($p > 0.05$). A decrease of Δ-ALA concentration in the urine of workers in 1999 *vs*. 2000 confirms a low level of lead dose in the body, since very similar data have been reported by other studies (Hisgashikawa et al., 2000; Makino et al., 2000; Molina-Ballesteros et al., 1976).

Despite a decrease of blood lead level and increased excretion of lead with urine, the Δ-ALAD activity in the blood of workers in 2000 decreased significantly compared to both the workers in 1998 and control group in 2000, $p < 0.001$ and $p < 0.001$, respectively. Lead is probably accumulated in the body, and even low lead doses have an adverse impact on its functioning and on the activity of haeme enzymes.

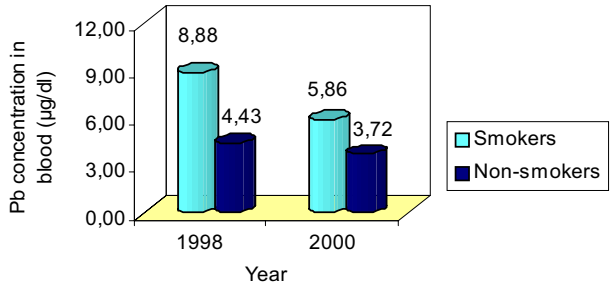

Fig. 1. Mean of blood lead level in smoking and non-smoking workers

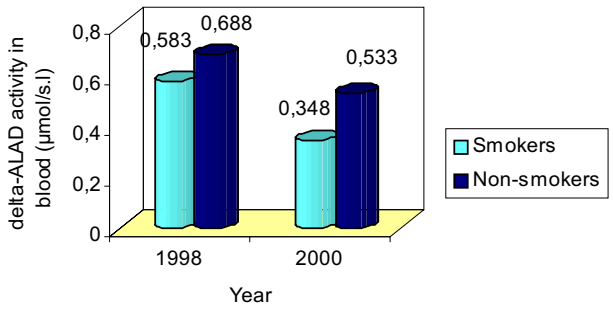

Fig. 2. Mean of blood Δ-ALAD activity in smoking and non-smoking workers

CONCLUSIONS

- Δ-ALAD activity in the blood of people occupationally exposed to lead decreases while Δ-ALA level in urine increases under the impact of lead and its compounds on the human body.
- Smoking increases lead burden in people occupationally exposed to lead.
- Even low levels of lead significantly decrease Δ-ALAD activity in blood. Δ-ALAD activity is one of the most sensitive biomarkers of the impact of low lead doses.

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ŠVINO POVEIKIO BIOŽYMEKLIŲ ĮVERTINIMAS ELEKTROS MONTUOTOJŲ-DERINTOJŲ TYRIMO KARTOTINĖJE STUDIJOJE

S a n t r a u k a

Delta-aminolevulino rūgšties dehidratazės (Δ-ALRD) aktyvumas kraujyje ir delta-aminolevulino rūgšties (Δ-ALR) koncentracija šlapime yra vieni jautriausių švino poveikio biožymeklių. Veikiant švinui Δ-ALRD aktyvumas kraujyje mažėja, o su šlapimu išsiskiriančios Δ-ALR kiekis didėja.

Šio darbo tikslas buvo įvertinti švino poveikio biožymeklių pokyčius AB „Lietuvos energija“ filialo „Kauno elektros tinklai“ žmonių (n = 13), dirbančių švinu užterštoje aplinkoje, organizme. Gauti duomenys palyginti su kontrole (n = 19).

1998 ir 2000 m. darbuotojams buvo tirtas Δ-ALRD aktyvumas ir Pb kiekis kraujyje, o 1999 ir 2000 m. nustatyta Δ-ALR ir Pb koncentracija šlapime. Švino kiekis matuotas ETG AAS Perkin-Elmer/Zeeman 3030. Δ-ALRD aktyvumas kraujyje ir Δ-ALR kiekis šlapime matuotas spektrofotometru. Statistinei duomenų analizei įvertinti naudotas priklausomų ir nepriklausomų parametrų t kriterijus.

Nustatytas Δ-ALRD aktyvumas darbuotojų kraujyje 2000 m. buvo statistiškai patikimai mažesnis už šį aktyvumą darbuotojų kraujyje 1998 m. ir kontrolinės grupės, atitinkamai $p < 0{,}001$ ir $p < 0{,}001$. Švino koncentracija darbuotojų kraujyje ir šlapime buvo statistiškai patikimai didesnė nei kontrolinės grupės žmonių, atitinkamai $p < 0{,}01$ ir $p < 0{,}01$. Nors nustatyta Pb koncentracija dirbančių švinu užterštoje aplinkoje žmonių kraujyje ir šlapime nebuvo didesnė už leistinas koncentracijas, tačiau nustatytas reikšmingas Δ-ALRD aktyvumo kraujyje sumažėjimas ir Δ-ALR kiekio šlapime padidėjimas, palyginti su kontroline grupe. Δ-ALRD aktyvumo kraujyje pokytis yra jautrus mažų švino dozių poveikio organizmui indikatorius.

Raktažodžiai: švino poveikio biožymekliai, delta-aminolevulino rūgšties dehidratazė (Δ-ALRD), delta-aminolevulino rūgštis (Δ-ALR), profesinis sąlytis su švinu