The Influence of the Occupational Exposure to Heavy Metals and Tobacco Smoke on the Selected Oxidative Stress Markers in Smelters

Milena Ściskalska · Marta Zalewska · Agnieszka Grzelak · Halina Milnerowicz

Received: 13 February 2014 / Accepted: 14 April 2014 / Published online: 1 May 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract The aim of the study was to verify if there is any association between exposure to Cu, Zn, Cd, Pb, As and the formation of malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), advanced oxidation protein products (AOPP), and whether in this process cigarette smoking plays a role. The investigations were performed in the 352 smelters occupationally exposed to heavy metals and 73 persons of control group. Metals concentration was determined by atomic absorption spectrometry. MDA and AOPP concentrations were determined by spectrophotometric methods. The concentration of 8-OHdG was determined by ELISA method. It was demonstrated an increased Cu concentration in smoking smelters compared to non-smoking control group. It was noted no differences in Zn and Mg concentrations between the examined groups. Pb concentration was more than sixfold higher in the group of smoking smelters and about fivefold higher in the group of non-smoking smelters compared to the control groups (smokers and non-smokers). Pb concentration was more than sixfold higher in the group of smoking smelters and about fivefold higher in the group of non-smoking smelters compared to the control groups (smokers and non-smokers). It was shown that Cd concentration in the blood was nearly fivefold higher in the smoking control group compared to the non-smoking control group and more than threefold higher in the group of smoking smelters compared to non-smoking. It was shown an increased As concentration (more than fourfold) and decreased Ca concentration in both groups of smelters compared to control groups. In groups of smelters (smokers and non-smokers), twofold higher MDA and AOPP concentrations, and AOPP/albumin index compared to control groups (smokers and non-smokers) were shown. Tobacco smoke is the major source of Cd in the blood of smelters. Occupational exposure causes accumulation of Pb in the blood. Occupational exposure to heavy metals causes raise of MDA concentration and causes greater increase in AOPP concentration than tobacco smoke.

Keywords AOPP · Cadmium · Heavy metals · MDA · Tobacco smoke · 8-OHdG

Introduction

Free radicals are not only formed continuously during normal physiological processes, but also as a result of external factors, including exposure to tobacco smoke in the case of smokers or to heavy metals in the work environment. In tobacco smoke, free radicals are responsible for cell damage and apoptosis [1]. They can cause DNA fragmentation, oxidative damage of cellular membrane lipids and proteins, and the reduction of enzyme activity through its oxidation and nitration [1–3]. The most important radicals, which have a strong toxic effect, are oxygen free radicals—superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), and nitrogen free radicals—peroxynitrites (NO$_2^–$) and nitrates (NO$_3^–$) [3]. Except to these radicals generated directly, tobacco smoke contains many substances, such as polycyclic aromatic hydrocarbons, heterocyclic substances, N-nitrosamines, aromatic amines, and aldehydes, which are probably the main causes of biomolecules damage [4, 5]. Components of tobacco smoke are also metals, e.g., arsenic, beryllium, nickel, chromium, cadmium, polonium, cobalt, and lead [4]. These
substances have the ability to generate free radicals or catalyze reactions in which they are produced [3].

Oxidative stress can intensify lipids peroxidation. One of the most common products of lipid peroxidation is malondialdehyde (MDA), which is also generated in the process of biosynthesis of prostaglandins. MDA can be transmitted to distant tissues and, thanks to the possibility of forming covalent bonds with other molecules, modifies their structure, and consequently, changes its properties. MDA has high cytotoxicity, is mutagenic, and inhibits the activity of various enzymes, leading to inhibition of DNA replication, transcription, and breathing [6, 7]. MDA is considered as a major and predominant antioxidant in plasma [18]. The ability of the albumin to perform these functions has a relationship with its structure, which may be destroyed by oxidative stress. Free radicals can interact with each other or with amino acids/proteins. The modifications of amino acids lead to an increase of carbonyl groups and disulphides in the protein molecule, and reduce the number of α-helices, what results in conformational changes of proteins [3, 19, 20]. The influence on the albumin function, in particular on its ability to transport, have not only free radicals, but also heavy metal ions. Their binding to albumin is in competition with other physiological substances transported by albumin. Toxic metals, as compounds with high affinity to the hydrophobic sites of albumin, cause a conformational change of the protein molecule, the amino acids oxidation, and the weakness of the ability to binding other ligands. In addition, the heavy metal ions present in the blood cause a chelation of physiological ligands. However, the strength of the phenomenon is dependent on the type of metal. In the presence of heavy metal ions, the toxins, which are transported by albumin, are displaced from the connections with protein. This results in an increase of free toxins in the blood, having an impact on the body [21, 22].

The oxidation of proteins, including albumin, leads to the formation of permanent products, which vary in terms of structure, function, and physicochemical properties. These are known as an advanced oxidation protein products (AOPP) [23]. Although the exact structure of AOPP is not completely understood, it is known that they are mainly derivatives of oxidatively modified albumin, its aggregates and/or fragments, but also fibrinogen and lipoproteins, glycoproteins, and globulins (e.g., thyroglobulin and γ-globulins) [18, 23–26].

The aim of the study was to verify if there is any association between exposure to metals (Cu, Zn, Cd, Pb, and As) occurring in the work environment of smelters and the formation of MDA, 8-OHdG, and AOPP and whether in this process cigarette smoking plays a role. The influence of the exposure to heavy metals and tobacco smoke on the concentration of Ca and Mg also was analyzed.

Material and Methods

Materials

The investigations were performed in the whole blood, serum, plasma, and urine of 352 males working in the copper foundry and 73 healthy men, non-exposed occupationally to heavy metals, who were qualified as control group by clinician of primary medical care. The study protocol was approved by Local Bioethics Committee of Wroclaw Medical University (Nr KB: 469/2008). The study population was in similar age and similar BMI. Table 1 presents data concerning age, BMI,
Heavy Metals and Tobacco Smoke Induce the Oxidative Stress

Table 1 Characteristics of the persons in control group and the group of smelters

|                         | Smoking X ± SD | Non-smoking X ± SD |
|-------------------------|----------------|-------------------|
| Control group (n=14)    |                |                   |
| Age                     | 34.38±7.90     | 36.64±11.49       |
| BMI [kg/m²]             | 26.98±5.48     | 25.75±3.50        |
| Years of work in smelter|                |                   |
| Serum cotinine [ng/ml]  | 66.46±12.18    | 3.46±3.47         |
| Smelters (n=258)        |                |                   |
| Age                     | 42.06±9.06     | 44.02±8.86        |
| BMI [kg/m²]             | 26.02±3.66     | 27.41±3.61        |
| Years of work in smelter| 18.98±1.87     | 19.99±8.82        |
| Serum cotinine [ng/ml]  | 71.19±12.65a   | 2.81±1.94         |

*p<0.0001 compared to non-smoking group

BMI body mass index

years of work in metallurgy, and cotinine concentration in serum of smelters and persons of control group divided into smokers (22 pack years of smoking) and non-smokers: 258 smoking smelters, 94 non-smoking smelters, 14 smokers of control group, and 59 non-smokers of control group. Data about smoking were obtained from direct personal interview and were verified by the determination of serum cotinine concentration—the metabolite of nicotine.

Venous blood was collected in the morning, after 12-h fasting. Serum was obtained according to the standard procedure by taking venous blood for disposable trace element-free tubes (No. Cat. 03.1524.001, Sarstedt, Germany) with serum clotting activator, left at 25 °C to complete thrombosis, and centrifuged (1,200 g/20 min). In order to obtain plasma, blood was collected into trace element-free tubes containing EDTA-K₂ (No. Cat. 04.1931.001, Sarstedt, Germany), immediately gently mixed, and centrifuged (2,500 g/15 min). The obtained serum, plasma, and whole blood were portioned and stored in sealed tubes (No. Cat. 0030102.002, Eppendorf, Germany). Blood samples of smelters and persons of control groups were stored at −80 °C until analysis.

The urine was collected in the morning, according to the standard procedure. Subjects were asked to wash their hands before supplying the urine, in order to reduce contamination. Urine samples were stored in special container containing liquid nitric acid at −20 °C until determination.

Metals Concentration

Concentrations of metals were determined using Solaar M6 apparatus (Solaar House, Cambridge, UK). The Pb concentration in whole blood (Pb-B) was determined by graphite furnace atomic absorption spectrometry (GFAAS) in the graphite Massman cuvette, the absorbance measurement at wavelength λ=283.3 nm, with Zieeman background correction. The reference material was used BCR-194, -195, -196, IRMM, EU. The Cd concentration in whole blood (Cd-B) and in urine (Cd-U) was determined by the same method at wavelength λ=228.8 nm, with Zieeman background correction. The concentration of Zn and Cu in serum (Zn-S and Cu-S) was determined by flame atomic absorption spectrometry (FAAS) in air-acetylene flame at wavelength λ=324.8 nm. The reference material was used Single-Element Zinc (Copper), standard 1000 µg/ml, CPI International. The determination of Ca and Mg concentrations in serum (Ca-S and Mg-S) was also performed by this method at wavelength λ=422.7 nm for Ca and λ=285.2 nm for Mg, with deuterium correction of the glass. As a reference material for these metals was used Seronorm™ Trace Element Serum (Sero, No. 201 405). The As concentration in urine (As-U) (with 65 % HNO₃ for acidification) was determined by FAAS method with snap Phillips PU 9360, used for hydride generation. In this method, wavelength λ=193.7 nm deuterium background correction and quartz atomization chamber were used.

Creatinine Concentration in Urine

Urinary creatinine level was determined by the Jaffé reaction [27].

Cotinine Concentration

Serum cotinine concentration was determined using a competitive enzyme immunoassay, the DRG Serum Cotinine ELISA kit (Cat. No. EIA-3242, DRG International, USA). The cotinine determination was carried out by the method described earlier [28, 29].

Plasma MDA Concentration (MDA-P)

Lipid peroxidation was measured in plasma by measuring the formation of thiobarbituric acid reactive substances (TBARS), quantified as malondialdehyde (MDA) equivalents, according to the method described earlier [30, 31]. The amount of MDA was calculated using an extinction coefficient (1.56×10⁵ M⁻¹ cm⁻¹). The MDA intensity was measured by spectrophotometric method at λ=535 nm. The concentrations of MDA were expressed as micromoles per liter in plasma.

Serum 8-OHdG Concentration (8-OHdG-S)

To evaluate the concentration of 8-OHdG in the serum, a commercial test OxiSelect™ DNA Oxidative Damage ELISA Kit was used (Cat. No. STA-320, Cell Biolabs, Inc., USA). To 96-well plate, 100 µl of conjugate 8-OHdG/BSA was added and incubated overnight at 4 °C, and then washed with H₂O. The next step, 200 µl blocking buffer was added and incubated at room temperature for 1 h. Fifty microliter of samples and
8-OHdG standards were added. After 10 min of incubation, monoclonal anti-8-OHdG was added (100 μl, 1 h incubation at room temperature), washed three times followed by addition of secondary antibody conjugated to horseradish peroxidase (100 μl, 1 h incubation at room temperature). The plate was washed three times with washing buffer and to each well 100 μl of substrate for peroxidase was added and incubated for 20 min. Followed by the addition of 100 μl of reaction stop solution. A spectrophotometric measurement of absorbance was performed at a wavelength λ=450 nm. The content of 8-OHdG in the tested samples was calculated by comparison with a standard curve determined from standards treated similarly to the samples tested.

Plasma AOPP Concentration (AOPP-P)

Determination of AOPP-P concentration was performed by spectrophotometric method developed by Witko-Sarsat et al. [26], based on the reaction of AOPP with potassium iodide in acidic conditions. To 1 ml of plasma, tenfold diluted with PBS, and to the blank sample (1 ml PBS), 50 μl of potassium iodide (99 %) and 100 μl of acetic acid (99.5 %) were added and mixed. The absorbance of samples was measured against the blank at wavelength λ=340 nm. The results of determination were expressed in micromoles per liter chloramine T equivalents and also were converted per gram of albumin and expressed as AOPP/albumin index (μmol/g).

Serum Albumin Concentration (Alb-S)

Alb-S concentration was determined by using the bromocresol purple (BCP) (Cat. No. 115–40–2, Sigma-Aldrich), which reacts with albumin in phosphate buffer saline (PBS) at pH=6.8. The concentration of albumin standard (Cat. No. 70024–90–70, Sigma-Aldrich) was determined using the molar absorption coefficient (A280 nm 0.1 % =0.5). To make the standard curve, the standard solutions at concentrations 0, 11.3, 22.6, 33.9, 45.2, 56.5 g/l were prepared, which were obtained by the dilution of albumin in PBS.

For each of the tubes, working reagent (with the final concentration of BCP 40 μmol/l), serum sample, or albumin standard were added and mixed. All samples were incubated for 5 min at 25 °C. Absorbances of the standards and of the samples were measured against the blank sample (working reagent) at wavelength λ=603 nm. The increase in absorbance was proportional to the concentration of albumin in the sample.

Statistical Analysis

Statistical analysis was carried out using the program Statistica 10.0. Data about examined groups, BMI, age, years of work in smeltery, and cotinine concentration, were analysed using ANOVA Kruskal-Wallis test. The concentration of metals (Cu-S, Zn-S, Pb-B, Cd-B, Cd-U, As-U, Ca-S, and Mg-S), MDA-P, 8-OHdG, AOPP-P, albumin, and the value of AOPP/albumin index also using the same test were analyzed. In order to verify the association between parameters (age, BMI, concentration of cotinine, metals, MDA-P, AOPP-P, albumin, and AOPP/albumin index), the multiple linear regression models were performed. Statistical significance was accepted for p<0.05.

Results

The Concentrations of Metals

In this study, the concentrations of Cu, Zn, Ca, and Mg in serum; Pb and Cd in whole blood; and Cd and As in urine were determined (Table 2). In the smoking smelters, a higher concentration of Cu in serum (Cu-S) compared to non-smoking control group was shown. There was no statistically significant difference in serum Zn (Zn-S) and Mg (Mg-S) concentrations between smoking and non-smoking smelters, and between smoking and non-smoking control group. In the serum of smoking and non-smoking smelters, the decrease of Ca concentration (Ca-S) in comparison to the smokers and non-smokers in control group was noted.

In both smoking and non-smoking smelters, a significant increase in the concentration of Pb in the blood (Pb-B) compared to the control group (smokers and non-smokers) was observed. In the group of smoking smelters, 6-fold increase in Pb-B concentration in comparison to the smoking control group and 7.4-fold higher in relation to the non-smoking control group were observed. In the group of non-smoking smelters, 5.7-fold higher Pb-B concentration compared to the non-smoking control group was observed. The concentration of Cd in the blood (Cd-B) was significantly higher at smokers (both the smelters and the control group) compared to non-smokers. In the blood of smokers (both the smelters and the control group), 3.3-fold higher Cd concentration compared to the non-smoking smelters and 4.8-fold higher compared to the non-smoking control group were observed.

In the group of smoking smelters, 2.5-fold higher concentration of Cd in urine (Cd-U) compared to the non-smoking control group was observed. In the urine of smoking and non-smoking smelters, As concentration (As-U) was significantly higher compared to control groups (both smokers and non-smokers). In the smelters (smoking and non-smoking), more than 4-fold higher of As-U concentration in comparison to the smoking control group and more than 4.6-fold higher compared to the non-smoking control group were observed.
MDA-P, 8-OHdG-S, and AOPP-P Concentrations as Markers of Lipids Peroxidation, Oxidatively Modified DNA, and Protein Oxidation

In the groups of smelters (smokers and non-smokers), 2-fold higher MDA-P concentration compared to control groups (respectively, smokers and non-smokers) was shown (Table 3). However, there was no statistically significant difference in 8-OHdG-S concentration between smoking and non-smoking smelters, and between smoking and non-smoking control group (Table 3).

In the plasma of smoking and non-smoking smelters, 2-fold higher of AOPP-P concentration in comparison to the smoking control group and 2.2-fold higher compared to the non-smoking control group was shown (Table 3). There were no statistically significant differences in Alb-S concentration between smoking and non-smoking smelters, and between smoking and non-smoking control group (Table 3).

AOPP/albumin index, which is expressed in micromole as the amount of AOPP per gram of albumin, was calculated. In the group of smoking smelters, 1.7-fold increase in the value AOPP/albumin index compared to the smoking control group and 3-fold increase of this parameter compared to the non-smoking control group was observed. 2.3-fold increase in the value of AOPP/albumin index in the non-smoking smelters compared to non-smoking control group was observed (Table 3).

Correlations

In the serum of non-smoking smelters, the correlation was demonstrated between the concentration of Cu-S and Alb-S ($r=-0.4646, p=0.0103$). The correlation of Pb-B and AOPP-P concentrations ($r=0.5219, p=0.0073$), and the AOPP/albumin index ($r=0.3689, p=0.0369$) in this group also were shown.

Discussion

Tobacco smoke and environmental or occupational exposure to heavy metals can cause many health effects in organism of copper foundry workers. Depending on intensity and duration of the exposure, they can change the biological functions of organs, such as liver and kidney. Lead, arsenic, and cadmium belong to toxic agents, which can disturb function of cardiovascular system. Heavy metals can produce free radicals, which leads to pro-oxidant/antioxidant imbalance and oxidative stress. It leads to the disturbances of cellular metabolism resulting in the formation of permanent changes caused by the oxidation in the structure of lipids, proteins, and DNA [32]. The oxidation products are MDA, AOPP and 8-OHdG respectively. The association of the MDA, 8-OHdG, and AOPP formation and oxidative stress was confirmed in subsequent studies [2, 18, 26, 33–38].

It was demonstrated that occupational exposure causes an increase in serum Cu concentration in smelters ($104.5\pm15.1 \mu g/%$) compared to controls ($99.7\pm12.1 \mu g/%$) [39]. Also, in our study, the highest Cu-S concentration in the group of smoking smelters was shown. This indicates that Cu-S concentration was increased with the number of oxidizing factors, especially during exposure to heavy metals. It confirmed that Cu can be replaced through heavy metals in

---

Table 2 Influence of cigarette smoking on the metals concentrations in the group of smelters and control group

| Metal   | Smelters          | Control group         |
|---------|-------------------|-----------------------|
|         | Smoking (n=258)   | Non-smoking (n=94)    |
|         | X±SD              | X±SD                  |
|         | Smoking (n=14)    | Non-smoking (n=59)    |
|         | X±SD              | X±SD                  |
| Cu-S   | 107.2±13.22b      | 104.00±13.63          |
| [μg/100 ml] |               | 102.29±13.46          |
| Zn-S   | 101.22±23.37      | 97.60±18.44           |
| [μg/100 ml] |               | 99.22±10.67           |
| Pb-B   | 229.91±127.75k,b  | 177.91±125.177k,b     |
| [μg/l] | 1.52±0.98k,b,     | 0.46±0.33a            |
| Cd-B   | 0.83±0.66k,b      | 0.56±0.41             |
| [μg/g creatinine] |      | 0.44±0.26             |
| Cd-U   | 12.76±7.69k,b     | 13.01±9.56k,b         |
| [μg/g creatinine] |      | 3.14±2.16             |
| As-U   | 89.81±8.03k,b     | 90.77±8.34k,b         |
| [μg/ml] |                   | 97.59±4.64            |
| Mg-S   | 21.08±2.01        | 20.62±1.10            |
| [μg/ml] |                   | 19.76±1.64            |
|        |                   | 20.70±1.70            |

---

$p<0.05$ compared to smoking control group

$b p<0.05$ compared to non-smoking control group

$c p<0.05$ compared to non-smoking smelters

Cu-S Cu concentration in serum; Zn-S Zn concentration in serum; Pb-B Pb concentration in the blood; Cd-B Cd concentration in the blood; Cd-U Cd concentration in urine; As-U As concentration in urine; Cu-S Ca concentration in serum; Mg-S Mg concentration in serum.
antioxidant enzymes, such as Cu/Zn superoxide dismutase (EC 1.15.1.1), which can result in the inhibition of antioxidant enzymes activity and an increase in oxidative stress [40]. On the other hand, in our study, there was no difference in Zn-S concentration in examined groups. It suggested the adaptation of healthy smelters to the environmental conditions, which was shown in others studies [41].

Bizoń et al. (2013) demonstrated that exposure to heavy metals causes an increase of Pb-B concentration [42]. Higher Pb-B concentration in the group of persons exposed to heavy metals (277.18±119.14 μg/l) compared to the control group (32.11±8.89 μg/l) was shown [42]. In other studies, a significant increase in Pb-B concentration in the group of smelters (201.2±112.5 μg/l) compared to the people not exposed to this metal (37.9±27.1 μg/l) was also shown [39]. In our study, a significant increase in Pb-B concentration in the groups of smelters compared to the control groups was observed. In the smoking smelters, the concentration of Pb was nearly sixfold higher in comparison to the non-smoking control group. It was also shown that Pb-B concentration in the group of smelters almost twofold exceeded the permissible level of this metal in the blood (<100 μg/l) [39, 43]. The Pb-B concentrations in the control groups were smaller than 100 μg/l. This confirms that the high Pb-B concentration was caused mainly by occupational exposure, and cigarette smoking was not the main source of Pb-B.

Cd-B and Cd-U concentrations increase during exposure to tobacco smoke, that was observed by other scientists. Bizoń et al. (2013) demonstrated an increase in Cd-B concentration of smokers compared to non-smokers, both in the control group and the group of smelters (0.43±0.39 μg/l, 1.59±0.79 μg/l and 1.69±0.70 μg/l, respectively, for the non-smoking control group and smoking control group <20 cigarettes/day and >20 cigarettes/day and 0.54±0.44 μg/l, 2.19±1.51 μg/l and 3.05±2.29 μg/l, respectively, for non-smoking and smoking smelters <20 cigarettes/day and >20 cigarettes/day) [42]. In other studies, the difference between Cd-B concentration in smokers (1.3 μg/l) and non-smokers (0.4 μg/l) was shown [44]. Madeddu et al. (2011) also demonstrated that cigarette smoking causes an increase of the Cd-B concentration (0.46 and 0.29 μg/l, respectively, for smokers and non-smokers) [45]. In this study, nearly fivefold higher of Cd-B concentration in the group of smokers compared to non-smokers (both smelters and the control group) was shown. Cd-B concentration in the group of smokers (both smelters and the control group) exceeded about threefold the value of permissible level (<0.5 μg/l) [39, 43]. In the group of non-smokers (both smelters and the control group), it did not exceed the permissible value. This emphasizes the importance of the influence of cigarette smoking on Cd-B concentration. It was observed higher Cd-U concentration in the smoking groups compared to non-smoking groups (both smelters and the control group). It was noted a higher Cd-U concentration in the group of smoking smelters compared to the smoking control group. It can suggest that occupational exposure to heavy metals accompanying exposure to tobacco smoke raised Cd-U concentration. This data confirmed that the main factor influencing on the Cd-B and Cd-U concentration is cigarette smoking. Occupational exposure to this metal seems

| **Table 3** Influence of cigarette smoking on MDA, 8-OHdG, AOPP, albumin concentrations, and AOPP/albumin index in the group of smelters and control group |
|----------------|----------------|----------------|----------------|
| **Parametr**   | **Smelters**   | **Control group** |
|                | Smoking (n=258) | Non-smoking (n=94) | Smoking (n=14) | Non-smoking (n=59) |
| MDA-P [μmol/l] | 1.56±0.76ab     | 1.54±0.65ab     | 0.74±0.12      | 0.80±0.24         |
| 8-OHdG-S [ng/ml]| 2.73±0.31       | 2.63±0.41       | 2.60±0.48      | 2.61±0.38         |
| AOPP-P [μmol/l]| 57.63±22.53ab   | 59.46±21.98ab   | 30.85±12.95    | 25.93±10.31       |
| Alb-S [g/l]    | 50.67±8.58      | 52.52±8.89      | 53.14±6.62     | 51.00±8.07        |
| AOPP/albumin index [μmol/g] | 1.09±0.50ab | 1.14±0.46ab | 0.64±0.18b | 0.49±0.17 |

* p<0.05 compared to smoking control group
b p<0.05 compared to non-smoking control group
c p<0.05 compared to non-smoking smelters

AOPP-P AOPP concentration in plasma; Alb-S Albumin concentration in serum; MDA-P MDA concentration in plasma; 8-OHdG-S 8-hydroxy-2'-deoxyguanosine concentration in serum
to have lower importance on its concentration in the human body than cigarette smoking.

An increase of As-U concentration in the group of persons occupationally exposed to heavy metals (18.9±22.2 μg/g creatinine) in comparison to the control group (3.5±3.0 μg/g creatinine) was observed [39]. Bizon et al. (2013) also have shown higher As-U concentration in smelters compared to the control group [42]. Our study confirmed the influence of occupational exposure on As-U, because in the groups of smelters (smokers and non-smokers) more than fourfold an increase in As-U concentration compared to the control groups was shown. It was also observed that in the group of smelters, As-U concentration exceeded the value of permissible level (<10 μg/g creatinine) [39].

The concentrations of Ca and Mg macroelements, which values have a significant impact on the reduction of the penetration capability of some toxic metals, such as Cd and Pb were also determined. The demonstrated differences in Ca-S concentration between the groups of smelters and control groups (both smokers and non-smokers) confirm that exposure to heavy metals causes a decrease Ca-S concentration. These results are similar to the study made by Kossowska et al., where was demonstrated a difference between Ca-S concentration in the group of smelters (90.4±7.3 μg/ml) and the control group (99.3±4.1 μg/ml) [39]. In our study, the influence of occupational exposure and cigarette smoking on Mg-S concentration was not observed. It is known that Mg has antioxidant properties, scavenging oxygen radicals, possibly by affecting the rate of spontaneous dismutation of the superoxide ion [46]. It was shown that Mg can inhibit lipoprotein oxidation and enhances the antioxidant enzymes activity, which reduces oxidative stress [47]. In our study, this effect was not observed. There was no difference in Mg-S concentration between examined groups. It can suggest that lipids peroxidation and protein oxidation in smelters were not mediated thought reduced level of Mg-S or its deficiency.

Chronic exposure to heavy metals causes their accumulation in tissues and has the influence on the human health [48–50]. It was observed an increase in the concentration of Pb-B, Cu-S, and As-U in the group of smelters, which confirms that the occupational exposure is the source of these metals. However, Cd-B concentration increases during exposure to tobacco smoke. Both occupational exposure to heavy metals and tobacco smoke intensify the oxidative stress. It results in the lipids peroxidation expressed as MDA production and/or protein oxidation and the formation of AOPP.

There was observed significantly higher plasma MDA levels (2.67±0.69 μM) of workers exposed to lead than those in the control group (1.23±0.61 μM) [51]. However, the results presented by Dursun et al. indicate that increased concentrations of lipid peroxidation products in the blood of persons exposed to lead is dependent not only on the concentrations of lead, but also on the age and time of exposure [51]. Other researches have also shown that MDA concentrations were elevated in the all heavy metal-treated groups compared to the control groups [52]. In this study, MDA concentrations in the group of smelters (both smokers and non-smokers) were nearly 2-fold higher compared to smoking and non-smoking control groups, which confirms that oxidative stress is generated in the result of occupational exposure in the smeltery. Increased concentration of MDA confirms the relationship between occupational exposure to heavy metals and the development of oxidative stress, and its effect on cell membranes. An increase in lipids peroxidation in smelters is a major sign of oxidative stress in the blood, which can result in the changes of tissues and organs.

Barbato et al. (2010) conducted a meta-analysis in environmental pollution exposed employees and in five studied groups, increase of 8-OHdG in the urine was observed, and in three studied group, there was no effect [53]. Garçon et al. (2007) studied the effects of chronic exposure to cadmium and lead on kidney function, they have also not observed differences in the concentration of the marker in the urine between smelters and control group (respectively, 12.7±5.6 and 12.4±5.9 μg/g creatinine) [54]. Wu et al. (2004) have not found any difference in the concentration of 8-OHdG in urine among smokers and people not exposed to tobacco smoke [11]. Musarrat et al. (1996) also found that adducts of 8-OHdG concentration does not correlate with smoking [55]. This study has shown no significant difference in the level of 8-OHdG concentration in none of group. This indicates that both cigarette smoking and metals exposure appear to have only a small effect on 8-OHdG level in the blood.

It should be noted that AOPP concentration depends on many physiological and environmental factors, which may influence on the examined population (age, body weight, sex, environmental pollution, cigarette smoking, and physical condition) [56–59]. The impact of additional factors, except tested factors, is so large that wrong classification to the control group or not adequate comparison of obtained results with these from other studies can make the results uncomparable. This is confirmed by the fact of the lack of standardized method for the determination of AOPP, which was noticed by other researchers [18]. In this study, examined groups were standardized in terms of sex, age, BMI, and environmental exposure to heavy metals.

There are some studies demonstrating the impact of cigarette smoking on the formation the changes in the structure of proteins. It was shown an increase in the amount of carbonyl groups in the molecules of proteins, which are the component of AOPP. A difference in the amount of carbonyl groups was shown between the group of persons, which were intensive smokers (4.00±1.31 nmol/mg protein) and the persons not exposed to tobacco smoke (0.80±1.94 nmol/mg protein) [14]. However, others researchers found no differences in concentration of carbonyl groups between current and former
smokers (17.9±2.9 nmol/ml plasma for both groups) [5]. Also was shown no difference in the concentration of carbonyl groups between persons, which were a long-term smokers (17.9±2.8 and 17.9±3.1 nmol/ml plasma, respectively, for smokers 30–45 years and >45 years), and the persons, which were smokers <30 years (17.7±2.7 nmol/ml plasma) [5]. In some studies, it was shown that the tobacco smoking has no influence on the structure of proteins; in others, it was shown that it can induce protein oxidation measured as the increase in the amount of carbonyl groups in their molecules. This indicates that the influence of tobacco smoking on the changes in proteins structure and the formation of AOPP are not clearly defined. In our study, it was shown no influence of tobacco smoke on an increase of AOPP-P concentration. However, AOPP-P concentration was more than twofold higher in the groups of smelters than in the control groups. This indicates an influence of occupational exposure on protein oxidation. Occupational exposure to heavy metals causes a significant increase in AOPP-P concentration.

Differences in this parameter resulting from the impact of tobacco smoke on protein oxidation were less visible than resulting from occupational exposure.  

Because of the fact that in the composition of AOPP is mainly the products of albumin’s oxidation; the determination of this protein in the serum of smelters was an important element of this study. On the concentration of AOPP, it has an influence not only in many factors discussed above, but also the concentration of albumin in the blood of the examined persons, which were in reference range (35–55 g/l) [60]. For this reason, the parameter better expressing the changes in AOPP concentration is its conversion per gram of albumin in the blood [61–62]. The expression of AOPP concentration as AOPP/albumin index in this study allowed to make the amount of AOPP independent from the inter-person variability in the concentration of albumin. This enables to better define an exposure to oxidation factors (heavy metals and tobacco smoke). In this study, the conversion of AOPP per gram of albumin permitted to detect a statistically significant difference in the AOPP-P concentration between smoking and non-smoking control group. This difference was not detected in the case of AOPP-P concentration expressed in micromoles per liter (using the same statistical test for both parameters). It was shown that the value of AOPP/albumin index increased with the exposure to factors causing the formation of AOPP (heavy metals and also tobacco smoke).

In this study, it was demonstrated that an important factor influencing AOPP-P concentration is occupational exposure to heavy metals, especially Cu. The inverse correlation between Cu-S and Alb-S concentration may indicate the participation of Cu in the formation of AOPP and its oxidation effect on albumin. In the group of non-smoking smelters, it was demonstrated the correlation between the concentration of Pb-B and AOPP-P, and the value of AOPP/albumin index. This may indicate that high Pb-B concentration is an oxidation factor for proteins. These results suggest that Cu and Pb play an important role in protein oxidation. Cu can generate free radicals by Fenton reaction. Additionally, Pb can replace Cu in antioxidant enzymes that causes an increase in serum Cu concentration and intensifies an oxidative stress.

In this study, it was confirmed the influence of heavy metals exposure on lipids peroxidation. It indicates that occupationally exposure can have an important role in lipid degradation and cell damage. It was not observed any effect of heavy metals exposure or cigarette smoking on oxidative modification of DNA. It was shown that cigarette smoking and occupational exposure to heavy metals have an influence on protein oxidation. These oxidizing factors of which the source is the environment seem to be important for destruction of protein molecule and limit its function.

Conclusions

In this study, it was confirmed that tobacco smoke is the main source of Cd in the blood of smokers. The occupational exposure on heavy metals has no influence on Cd-B and Cd-U concentrations. With the increasing exposure to tobacco smoke and heavy metals, Ca concentration in serum was decreased.

Exposure to heavy metals causes an increase of heavy metals concentrations in the blood, especially Pb-B, but also Cu-S and As-U of smelters. This oxidizing factor intensifies lipids peroxidation and causes an increase in MDA concentration, but they have no influence on DNA. The intensity of AOPP formation depends on the type of factor that it causes—for smelters, occupationally exposure to heavy metals is a stronger oxidation factor than tobacco smoke. The strongest oxidation effect on the protein has Cu and Pb.

Acknowledgments This work was supported by the grant from Wroclaw Medical University. The authors gratefully thank Professor Jolanta Antonowicz-Juchniowicz from Department of Internal and Occupational Medicine, Wroclaw Medical University, Poland for her support in biological material collection.

Conflict of interest statement The authors declare that they have no conflict of interest.

Statement All persons gave their informed consent prior to their inclusion in the study.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Asano H, Horinouchi T, Mai Y, Sawada O, Fujii S, Nishiya T, Minami M, Katayama T, Iwana T, Terada K, Miwa S (2012) Nicotine- and tar-free cigarette smoke induces cell damage through
reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. J Pharmaco 64:257–257
2. Valavanidis A, Vlachogianni T, Fiokakis K (2009) Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respiratory particles. Int J Environ Res Public Health 6:445–462
3. Weina D, Khanin EV, Levy Y, Rzepk AZ (2009) Effects of cigarette smoke on reactive nitrogen species on salivary α-amylase activity and protein modifications. J Physiol Pharmacol 60:127–132
4. Starek A, Podolak I (2009) Carcinogenic effect of tobacco smoke. Rozc Panstw Zakl Hig 60:299–310
5. Yeh C, Barr RG, Powell CA, Mesia-Vela S, Wang Y, Hamade NK, Austin JHM, Santella RM (2008) No effect of cigarette smoking dose on oxidized plasma proteins. Environ Res 106:219–225
6. Klaunig JE, Wang Z, Pu X, Zhou S (2011) Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol Appl Pharmacol 254:86–99
7. Valavanidis A, Vlachogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 64(2):178–189
8. He X, Nie X, Wang Z, Cheng Z, Li K, Li G, Hung Wong M, Liang X, Tsui MT (2011) Assessment of typical pollutants in waterborne by combining active biomonitoring and integrated biomarkers response. Chemosphere 84:1422–1431
9. Zhang XY, Tan YL, Haile CN, Wu KY, Cao LY, Kosten TA, Kosten TR (2007) Nicotine dependence, symptoms and oxidative stress in male patients with schizophrenia. Neuropsychopharmacology 32:2020–2024
10. Delaney S, Jarem DA, Volle CB, Yennie CJ (2012) Chemical and biological consequences of oxidatively damaged guanine in DNA. Free Radic Res 46:420–441
11. Wu L, Chiu CC, Chang PY, Wu JT (2004) Urinary 8-OhD2: a marker of oxidative stress and DNA and a risk factor for cancer, atherosclerosis and diabetes. Clin Chim Acta 339:1–9
12. Valavanidis A, Vlachogianni T, Fiokakis K (2009) 8-hydroxy-2′-deoxyguanosine (8-OhdG): a critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:120–139
13. Pelger A, Rüdiger HW (2006) 8-Hydroxy-2′-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. Int Arch Occup Environ Health 80:1–5
14. Pignatelli B, Li CQ, Boffetta P, Chen Q, Ahrens W, Nyberg F, Mukeria A, Bruske-Hohlfeld I, Fortes C, Constantinescu V, Santangelo F, Descamps-Latscha B (2003) AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: a potential system of the plasma proteome? Free Radic Res 37:441–457
15. Zampella G, Neupane KP, De Gioia L, Pecoraro VL (2012) The importance of stereochemically active lone pairs for influencing Pb II and As III protein binding. Chem Eur J 18:2040–2050
16. Herman DS, Geraldine M, Venkatesh T (2009) Influence of minerals on lead-induced alterations in liver function in rats exposed to long-term lead exposure. J Hazard Mater 166:1410–1414
17. Goyet RA (1993) Lead toxicity: Current concerns. Environ Health Perspect 100:177–187
18. Selmesi L (2011) Advanced oxidation protein products (AOPP): Novel uricemic toxins, or components of the non-enzymatic antioxidant system of the plasma proteome? Free Radic Res 45:1115–1125
19. Annari M, Yamasaki K, Maruyama T, Kragh-Hansen U, Otugri M (2001) Effect of oxidative stress on the structure and function of human serum albumin. Farm Res 18:632–639
20. Iwao Y, Annari M, Yamasaki K, Kragh-Hansen U, Kawai K, Maruyama T, Otugri M (2006) Oxidation of Arg-410 promotes the elimination of human serum albumin. Biochim Biophys Acta 1764:743–749
21. Peng M, Shi S, Zhang Y (2012) The influence of Cd2+, Hg2+ and Pb2+ on taxifolin binding to bovine serum albumin by spectrospecc 22. Zhang Z, Shuyun S, Liu Y, Chen X, Pen M (2011) Differential effects of Cu (II) and Fe (III) on the binding of omeprazole and pantoprazole to bovine serum albumin: Toxic effect of metal ions on drugs. J Farm Biomed Anal 56:1064–1068
23. Liu B, Hou X, Zhou Q, Tian J, Zhu P, Xu J, Hou F, Fu N (2011) Detection of advanced oxidation protein products in patients with chronic kidney disease by a novel monoclonal antibody. Free Radic Res 45:662–671
24. Capellière-Blandin C, Gausson V, Descamps-Latscha B, Witko-Sarsat V (2004) Biochemical and spectrophotometric significance of advanced oxidized protein products. Biochem Biophys Acta 1698:91–102
25. Selmesi L, Székely M, Soós P, Seres L, Klinga N, Geiger A, Acsády G (2006) Human blood plasma advanced oxidation protein products (AOPP) correlates with fibrogenin levels. Free Radic Res 40:952–958
26. Witko-Sarsat V, Friedlander M, Capellière-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingriff J, Jungs P, Descamps-Latscha B (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 49:1304–1313
27. Butler AR (1975) The Jaffe reaction. Identification of the coloured species. Clin Chim Acta 59:227–232
28. Bizoh A, Milnerowicz-Nazdzyk E, Zalewska M, Zimmer M, Milnerowicz H (2011) Changes in pro/antioxidant balance in smoking and non-smoking pregnant women with intrauterine growth restriction. Reprod Toxicol 32:360–367
29. Zalewska M, Królik M, Milnerowicz H (2011) Impact of working in metallurgy and cigarettes smoking on the concentration of malonyldialdehyde and 8-hydroxydeoxyguanosine in the blood. Przegl Lek 68:770–774
30. Bouki E, Dimitriadius VK, Kaloyianni M, Dailianis S (2013) Antioxidant and pro-oxidant challenge of tannic acid in mussel hemocyes exposed to cadmium. Mar Environ Res 85:13–20
31. Chatziariyov V, Dailianis S (2010) The role of selenium-dependent glutathione peroxidase (Se-GPx) against oxidative and genotoxic effects of mercury in haemocytes of mussel Mytilus galloprovincialis (Link.). Toxicol In Vitro 24:1363–1372
32. Hansanad M, Omdal R, Norheim KB, Gøransson LG, Brede C, Jonsson G (2012) Improved detection of advanced oxidation protein products in plasma. Clin Chim Acta 413:901–906
33. Bulut M, Selek S, Beş Y, Kayaa MC, Gunes M, Karababac F, Celikd, H, Savas HA (2013) Lipid peroxidation markers in adults with deficiency in hyperactivity disorder: new findings for oxidative stress. Psychiatry Res 209:638–642
34. Guo CH, Chen PC, Lin KP, Shih MY, Ko WS (2012) Trace metal imbalance associated with oxidative stress and inflammatory status in an-shelitis C virus antibody positive subjects. Environ Toxicol Pharmacol 33:288–296
35. Hawkins CL, Pattison DI, Davies MJ (2003) Hypochlorite-induced oxidation of amino acids, peptides and proteins. Amino Acids 25:259–274
36. Jomova K, Valko M (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283:65–87
37. Piotowar A, Knapik-Kordecka M, Warwas M (2007) AOPP and its role in dialysis patients. Kidney Int 671:192–205
38. Antonowicz-Juchniewicz J (2010) Proteomic analysis of serum of heavy metals and tobacco smoke induce the oxidative stress.
49. Zhang X, Yang L, Li Y, Li H, Wang W, Ye B (2012) Impacts of heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res 138:501–523

50. Flora SJS, Mittal M, Mehta A (2008) Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res 138:501–523

51. Dursun N, Dogan P, Dommez H (2001) Plasma and erythrocyte lipid peroxide levels in workers with occupational exposure to lead. Biol Trace Elem Res 82:29–34

52. Turkcz H, Geyikoglu F, Tatar A, Keles MS, Kaplan I (2012) The effects of some boron compounds against heavy metal toxicity in human blood. Exp Toxicol Pathol 64:93–101

53. Barbato DL, Tomei G, Tomei F, Sancini A (2010) Traffic air pollution and oxidatively generated DNA damage: can urinary 8-oxo-7,8-dihydro-2-deoxyguanosine be considered a good biomarker? A meta-analysis. Biomarkers 15:538–545

54. Garçon G, Leleu B, Marez T, Zerimech F, Hagueneoum HM, Furon D, Shirali P (2007) Biomonitoring of the adverse effects induced by the chronic exposure to lead and cadmium on kidney function: Usefulness of alpha-glutathione S-transferase. Sci Total Environ 377:165–172

55. Musarrat J, Arnezina-Wilson J, Wani AA (1996) Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis. Eur J Cancer 32A:1209–1214

56. Çakatay U, Kayrali R, Uzun H (2008) Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. Clin Exp Med 8:51–57

57. Chirico EN, Martin C, Faës C, Féasson L, Oyono-Enguéllé S, Aufradet E, Dubouchaud H, Francina A, Canet-Soulaes E, Thiriou P, Messonnier L, Pialoux V (2012) Exercise training blunts oxidative stress in sickle cell trait carriers. J Appl Physiol 112:1445–1453

58. Selmei L, Seres L, Antal M, Lukács J, Regőly-Mérei A, Acsády G (2005) Advanced oxidation protein products (AOPP) for monitoring oxidative stress in critically ill patients: a simple, fast and inexpensive automated technique. Clin Chem Lab Med 43:294–297

59. Venturini D, Salmi A, Anrep G, Melo PA, Belinetti FM (2012) Evaluation of oxidative stress in overweight subjects with or without metabolic syndrome. Obesity 20:2361–2366

60. Dembińska-Kieć A, Drożdż R, Naskalski J (2010) Plasma proteins. In: Dembińska-Kieć A, Naskalski J (eds) Diagnostic Laboratory with the elements of clinical biochemistry, 3rd edn. Elsevier Urban & Partner, Wrocław, p 200

61. Matteucci E, Giampietro O (2000) Oxidative stress in families of type 1 diabetic patients. Diabetes Care 23:1182–1186

62. Šebeková K, Klenovíková K, Ferenczová J, Hedwig J, Podráká L, Heidland A (2012) Advanced oxidation protein products and advanced glycation end products in children and adolescents with chronic renal insufficiency. J Ren Nutr 22:143–148