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# Biomarkers of selenium status and antioxidant effect in workers occupationally exposed to mercury

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ABSTRACT

The present observation based research was designed to evaluate the influence of occupational human exposure to metallic mercury (Hg°) vapor on the biomarkers of selenium status involved in the antioxidant defense system. For this purpose we determined Hg and selenium (Se) concentrations in body fluids, the markers of antioxidant effect measured as an activity of Se-dependent enzymes (red blood cell and plasma glutathione peroxidase: GPx1-RBC and GPx3-P), concentration of selenoprotein P in the plasma (SeP-P) and total antioxidant activity in the plasma (TAA-P) in 131 male workers from a chloralkali plant exposed to Hg° and 67 non-exposed males (control group). The mRNA expression levels of glutathione peroxidases (*GPX1, GPX3*), selenoprotein P (*SEPP1*), thioredoxin reductase 1 (*TRXR1*), thioredoxin 1 (*TRX1*), peroxiredoxins (*PRDX1, PRDX2*) were also examined in the leukocytes of peripheral blood. Hg concentration in the blood (Hg-B) and urine (Hg-U) samples was determined using the thermal decomposition amalgamation/atomic absorption spectrometry (TDA-AAS) method and Se concentrations in plasma (Se-P) and urine (Se-U) using the inductively coupled plasma mass spectrometry (ICP-MS) method. Activities of GPx1-RBC, GPx3-P and TAA-P were determined using the kinetic and spectrophotometric method, respectively. Gene expression analysis was performed using the quantitative Real-Time PCR.

The results showed significant higher Hg levels among the Hg°–exposed workers in comparison to control group (12-times higher median for Hg-B and almost 74-times higher median for Hg-U concentration in chloralkali workers). Se-P was also significantly higher (Me (median): 82.85 μg/L (IQR (interquartile range) 72.03–90.28 μg/L) for chloralkali workers vs. Me: 72.74 μg/L (IQR 66.25-80.14 μg/L) for control group; $p = 0.0001$) but interestingly correlated inversely with Hg-U in chloralkali workers suggesting depletion of the Se protection among the workers with the highest Hg-U concentration. The mRNA level for *GPX1, PRXD1* were markedly but significantly higher in the workers compared to the control group. Moreover, concentrations of Hg-B and Hg-U among the workers were significantly positively correlated with the levels of selenoprotein P at both the mRNA and selenoprotein levels. In the multivariate model, after adjusting to cofounders (dental amalgam fillings, age, BMI, job seniority time, smoking), we confirmed that Hg-U concentration was inversely correlated with genes expression of *TRXR1*.

This is the first comprehensive assessment of the impact of occupational exposure of workers to Hg° at both the mRNA and selenoprotein levels, with investigation of fish intake obtained by means of a questionnaire. These findings suggest that exposure to Hg° alters gene expression of the antioxidant enzymes and the level of Se-containing selenoproteins.

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## 1. Introduction

Mercury (Hg) has adverse health effects, especially when it is inhaled as a vapor (Hg°). As a result of occupational exposure, Hg° is absorbed into the bloodstream and next converted to a more reactive $Hg^{2+}$ form. Toxic effect of Hg at the molecular level is primarily due to the formation of stable Hg complexes with sulfur present in the thiol groups and selenium (Se) in the selenol groups of proteins and other molecules of major biological significance (e.g.selenoprotein P, thioredoxin reductase and glutathione) [1,2]. It can also interact with endogenous sulfur-containing biomolecules: metallothionein and albumin forming complexes. They easily pass through cell membranes and then are distributed throughout the body and/or reabsorbed into proximal convoluted tubules or eliminated with the urine.

Effects of reduction of Se bioavailability (e.g.after formation of the mercury-selenoprotein complexes) in the bloodstream may then impair selenoprotein synthesis and its expression [3,4]. Moreover, inhibition glutathione peroxidase activity simultaneously prevents incorporation of Se into the active center, thus the enzyme becomes inactive [5,6]. A similar situation can be observed in the case of thioredoxin reductase – the next substantial Se-dependent enzyme, which has also an extremely important role in Hg-induced redox reactions [7].

Only few papers deal with the impact of occupational exposure to Hg° on selenoproteins levels involved in the antioxidant defense system [6,8–13]. To fill this gap, we evaluated impact of exposure to Hg via inhalation in the context of Hg-Se interaction through the analysis of Se-biomarkers of the antioxidant effect, including expression and/or activity of selected selenoproteins in chloralkali workers and control groups. It is important to note that our study, which was conducted on a larger population than any other study's and related to fish-eating habits, is the first comprehensive assessment of the impact of occupational exposure to Hg° at both the mRNA and selenoprotein levels.

## 2. Materials and methods

### 2.1. Study design

The study was conducted between May and June 2015 on the male workers (n = 131) with median (Me) age of 41 years, the interquartile range (IQR) 31–51 years from a chemical (chloralkali) industrial plant in Poland. Median of BMI was 27.1 $kg/m^2$ (IQR 24.3–29.8 $kg/m^2$). Median of occupational Hg° exposure time in the same workplace was 4.0 years (IQR 1.5–15.0 years) and in the whole plant 15.0 years (IQR 5.0–30.0 years). Because the occupationally exposed to Hg° group was exclusively male, the non-exposed men (control group) were well matched in terms of age, BMI (n = 67, $Me_{age}$: 37 years, (IQR 30–54 years), $Me_{BMI}$: 26.6 $kg/m^2$ (IQR 24.3–29.0 $kg/m^2$) and geographical region. The overall mean Hg° concentration in the air was 40 ± 0.007 $\mu g/m^3$ in the range from 34 $\mu g/m^3$ to 47 $\mu g/m^3$ (static air sampling, which is twice as high as the value of maximum allowable concentration (MAC) for occupational exposure. This result was delivered by a Health and Safety representative of the studied chemical plant. Characteristics of the studied groups and comparison of the measured parameters between the chloralkali workers and the control group are presented in Table 1. In order to obtain basic data needed for the research, a personal survey as well as validated Food Frequency Questionnaire (FFQ-6) [14] were conducted. The personal survey included questions concerning age, body mass index (BMI), current smoking status, alcohol, medical history, dental amalgam fillings. The semi-quantitative FFQ-6 was used for investigation of the frequency (times/person/day) of the food consumed during a year. All the participants were informed about the aim of the study and they provided their informed consent. The study was approved by the Ethics Committee of the Nofer Institute of Occupational Medicine in Lodz, Poland.

### 2.2. Specimen collection

The blood was collected into BD Vacutainer® Blood Collection Tubes (free from trace elements) containing Lithium Heparin as an anticoagulant. The heparinized blood samples were then centrifuged (10 min, 1500 × *g*, 4 °C) to obtain plasma and stored at −20 °C until the analysis. Erythrocytes were washed with a 0.9% solution of NaCl and hemolyzed three times by thawing and freezing. The urine samples were collected into disposable containers (previously cleaned in accordance with the washing procedures protecting against Hg contamination) and acidified to pH < 1.

### 2.3. Determination of Hg and Se in the biological material

Total mercury (THg) concentrations in the urine and blood samples collected from the chemical-plant workers were determined using the Thermal Decomposition Amalgamation/Atomic Absorption Spectrometry method (TDA-AAS) using Direct Mercury Analyzer (DMA-80 by Milestone, Spectro-Lab, Poland). Absorption intensity was measured at 253.7 nm. Continuous flow of oxygen transports decomposition products through a catalyst bed, which eliminates interferences.

Concentrations of Se-P (diluted one hundred fiftyfold with 1.0% $HNO_3$) and Se-U (diluted one tenth with 1.0% $HNO_3$) were determined using the inductively coupled plasma mass spectrometry ICP-MS (Elan DRC-e, Perkin Elmer, SCIEX, USA).

As regards determinations of total Hg and Se in the biological samples, the laboratory in charge of it has participated in the German External Quality Control (G-EQUAS) organized by the Institute of Occupational Social and Environmental Medicine of the University of Erlangen, Nuremberg.

### 2.4. Assay of activity of GPx1 and GPx3

Cytosolic GPx (cGPx, GPx-1) and plasma GPx (pGPx, GPx-3) activities were assayed according to the method of Paglia and Valentine [15] with t-butyl hydroperoxide as a substrate. GPx activity was measured by decreasing in NADPH absorbance measured at 340 nm during NADPH oxidation to $NADP^+$, using the Unicam UV4 UV–vis spectrophotometer (Cambridge, UK). Enzyme activities were expressed in units per gram of hemoglobin (GPx1) or mL of plasma (GPx3). The intra-assay coefficient of variability (CV) amounted 5%.

### 2.5. Assay of SeP-P concentrations

Selenoprotein P concentration in the plasma was measured using the immunochemical Sandwich ELISA test (USCN Life Science Inc kit, Hu, China). After pre-coating plate wells with a target specific capture antibody, all the diluted plasma samples (500–1000 times) were added to the wells. Next, a biotin-conjugated detection polyclonal antibody specific for SeP, which binds to the captured antigen, was placed, and next an avidin-conjugated horseradish peroxidase, which binds to the biotin was added. In the end, 3,3′,5,5′-tetramethylbenzidine substrate which reacts with the enzyme, was added resulting in color development. After adding a sulfuric acid stop solution, the optical density was measured at a wavelength of 450 nm.

### 2.6. Assay of TAA concentration

The pool of low molecular weight antioxidants in plasma was determined using the spectrophotometric method. After using the Antioxidant Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) the coloured end product was measured against the reagent blank at 430 nm (spectrophotometer MultiScan GO, Thermo Scientific, Waltham, MA, USA).

**Table 1**
Characteristic of studied groups and comparison of measured parameters between chloralkali workers and control group. Statistically significant p-values are shown in bold.

| Parameters | Chloralkali workers (n = 131) | | Control group (n = 67) | | P |
|---|---|---|---|---|---|
| | Median | (IQR) | Median | (IQR) | |
| Age | 41.00 | (31.00–51.00) | 37.00 | (30.00–54.00) | 0.7009 |
| BMI (kg/m$^2$) | 27.12 | (24.31–29.80) | 26.60 | (24.30–28.98) | 0.3487 |
| Duration of exposure to Hg° (in the workplace) (years) | 4.00 | (1.50–15.00) | – | – | NA |
| Duration of exposure to Hg° (in the plant) (years) | 15.00 | (5.00–30.00) | – | – | NA |
| The number of dental amalgam fillings | 0.00 | (0.00–1.00) | 0.00 | (0.00–1.00) | 0.8976 |
| Hg-B (μg/L) | **6.06** | **(4.01–8.94)** | **0.52** | **(0.35–0.73)** | **< 0.0001** |
| Hg-U (μg/g creat.) | **11.84** | **(7.55–21.04)** | **0.16** | **(0.12–0.24)** | **< 0.0001** |
| GPx3-P (U/mL) | 0.18 | (0.16–0.20) | 0.18 | (0.17–0.21) | 0.4566 |
| GPx1-RBC (U/gHb) | 22.46 | (19.30–25.37) | 22.64 | (19.31–25.03) | 0.8227 |
| SeP-P (μg/L) | 5.83 | (3.10–13.39) | 7.64 | (3.91–12.35) | 0.2122 |
| TAA-P (mmol/L) | **1.98** | **(1.78–2.10)** | **1.84** | **(1.62 - 1.99)** | **0.0038** |
| Se-P (μg/L) | **82.85** | **(72.03–90.28)** | **72.74** | **(66.25–80.14)** | **0.0001** |
| Se-U (μg/g creat.) | **13.44** | **(11.53–16.65)** | **11.89** | **(9.89–14.94)** | **0.0271** |

Mann-Whitney's test. NA – Not Applicable, IQR – The interquartile Range.

### 2.7. Gene expression analysis

Total RNA was isolated from the venous blood using PAXgene RNA Blood Mini Kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland). *GPX1*, *GPX3*, *SEPP1*, *TRXR1*, *TRX*, *PRDX1*, *PRDX2* gene expression in the peripheral blood leukocytes was analyzed using the quantitative Real-Time PCR with Universal Probes (Roche, Basel, Switzerland). Primers (Table 2) were designed using the Universal ProbeLibrary Assay Design Center (Roche, Basel, Switzerland). Based on the Human Reference Gene Panel (Roche, Basel, Switzerland), beta-actin (*ACTB*) and beta-2-microglobulin (*β2M*) were selected out of 19 candidate reference genes, as the most stable genes across all the samples.

The cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland). All the samples were amplified in duplicate. Expression was quantified with the FastStart Essential DNA Probes Master (Roche, Basel, Switzerland) using the LightCycler® 96 System (Roche, Basel, Switzerland). Gene expression data was evaluated by the dCt method a reference genes-normalized relative quantification. To determine the inter-assay variability, amplification for randomly selected samples was repeated within runs. The inter-assays CV for each of 7 genes were below 12% and the intra-assay CV was below 5%.

### 2.8. Determination of creatinine

Creatinine (creat.) in the urine was measured at a wavelength of 520 nm using the UV–vis spectrometer (Unicam UV4, Alva, United Kingdom) in accordance with the method with picric acid (Jaffe reaction) [16].

### 2.9. Food frequency questionnaire

For investigation of the frequency of the food consumed in a year, the FFQ-6 was used. Although we collected answers concerning intake of 62 assortment of products, we chose only one group of food including fish (low- and high-fat fish) and meat products (sausages, game, red meat, minced meat, rabbit meat). An individuals pointed habitual intake frequency in open questions, with a six-point scale: (1) never or nearly never, (2) once a month or less, (3) several times a month, (4) several times a week, (5) every day, (6) several times a day.

### 2.10. Statistical analysis

Continuous variables were presented as median with an interquartile range. Comparisons between the chloralkali workers and control groups in terms of continuous variables and differences between quantified usual dietary fish intake measured by FFQ-6 were performed by the use of the Mann-Whitney's test. Correlation coefficients were calculated using the Spearman rank test. Generalized linear models were built for the analysis of the relationships between the selected gene expression and Hg° exposure marker and confounding factors. The statistical analysis was conducted using STATISTICA 13.1 PL (StatSoft, Tulsa, OK, USA). Significance was established at a level of $p \leq 0.05$.

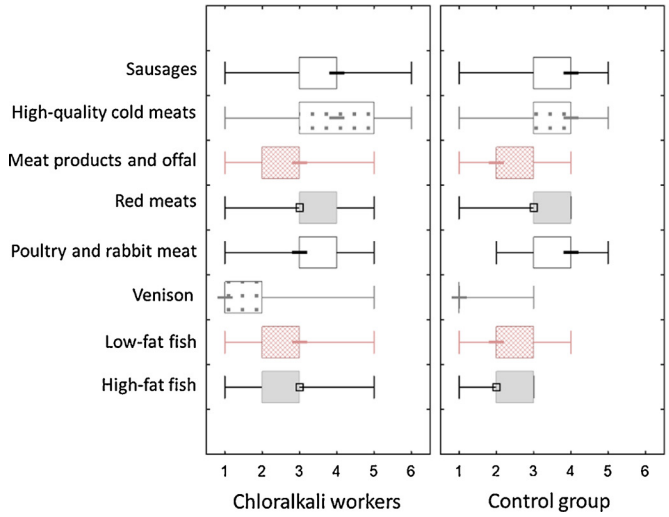
## 3. Results

### 3.1. Food frequency questionnaire

The analysis of distribution of food consumption frequency among examined individuals with regard to fish and meat products category is presented in Fig. 1. As we did not detected any strong, significant differences in terms of the intake of fish and meat products we assumed

**Table 2**
qRT-PCR primers for selenoproteins used in the present study.

| Gene name | Sense primer (5'–3') | Antisense primer (5'–3') | Universal Probe Library probe |
|---|---|---|---|
| *GPX1* | CAACCAGTTTGGGCATCAG | GTTCACCTCGCACTTCTCG | #77 |
| *GPX3* | GGGGACAAGAGAAGTCGAAGA | GCCAGCATACTGCTTGAAGG | #52 |
| *SEPP1* | GGAGCTGCCAGAGTAAAGCA | ACATTGCTGGGGTTGTCAC | #38 |
| *PRDX1* | CACTGACAAACATGGGGAAGT | TTTGCTCTTTTGGACATCAGG | #20 |
| *PRDX2* | CAGACGAGCATGGGGAAG | ACGTTGGGCTTAATCGTGTC | #11 |
| *TRXR1* | ACACAAAGCTTCAGCATGTCA | CAATTCCGAGAGCGTTCC | #81 |
| *TRX* | GGGACAAAAGGTGGGTGA | GCCAATGGCTGGTTATGTTT | #50 |
| *ACTB* | CCAACCGCGAGAAGATGA | CCAGAGGCGTACAGGGATAG | #64 |
| *B2M* | TTCTGGCCTGGAGGCTATC | TCAGGAAATTTGACTTTCCATTC | #42 |




| Variable | *p* |
|---|---|
| Sausages | 0.6564 |
| High-quality cold meats | 0.9134 |
| Meat products and offal | 0.4697 |
| Red meats | 0.0856 |
| Poultry and rabbit meat | 0.2230 |
| Venison | 0.4190 |
| Low-fat fish | 0.2212 |
| High-fat fish | 0.0744 |
| Meat products and fish [rank sum] | 0.8558 |

**Fig. 1.** An analysis of the distribution of frequency of food consumption in fish and meat categories.

that difference in Hg-exposure between the groups is rather due to occupational not environmental exposure difference.

### 3.2. Mercury in the biological material

Median Hg-U concentration in the chloralkali workers was almost 74-fold higher than it was in the control group and the difference was statistically significant ($p < 0.0001$) (Table 1). Moreover, 16% of the obtained results of the mean Hg-U concentrations among the workers exceeded the value applying in Poland for the biological exposure indices (the BEI value, Hg-U 30 µg/g creat.) and ranged from 1.01 to 105.53 µg/g creat. Median value of the measured Hg-B among Hg°–exposed workers was 12-times higher in comparison with that of the control group and was statistically significant ($p < 0.0001$). Six percent of the results of the mean Hg-B concentrations among the workers exceeded the BEI value for blood samples (Hg-B 15 µg/L) and ranged from 0.67 to 57.52 µg/L.

### 3.3. Selenium in the biological material

Plasma selenium median concentration in the assayed biological material from the chloralkali workers was 14% higher compared to the control group ($p = 0.0001$) (Table 1). Urine selenium concentration was higher by 13% ($p = 0.0271$). Moreover, we found a strong positive correlation between Se-P and Se-U concentrations ($R = 0.50$; $p < 0.0001$) and between Se-P and activity of GPx3 ($R = 0.38$; $p < 0.0001$) only in the Hg°-exposed workers group. Among the control group the correlation coefficient we were very small ($R = 0.08$, $p = 0.5257$ and $R = 0.18$, $p = 0.1598$) for Se-P vs. Se-U correlation and Se-P vs. GPx3 correlation, respectively but the direction the relationship was preserved between the groups.

Moreover, we showed a statistically significant, positive correlation between plasma selenium and expression of TRXR1 but only in the occupationally exposed group ($R = 0.24$; $p = 0.006$).

### 3.4. Selenium and mercury molar ratios

The difference in the calculated molar ratio (calculated using the mean Hg and Se values) between plasma (Hg in blood plasma Hg-P) Me $_{\text{Se:Hg in Hg-P}}$: 17.38 (IQR 9.18–21.06) and urine concentrations of the individuals occupationally exposed to Hg° (Me $_{\text{Se:Hg in Hg-U}}$: 1.10 (IQR 0.58–1.92) was 11- and 45-times (for plasma and urine respectively) greater than that in the control group (Me $_{\text{Se:Hg in Hg-P}}$: 190.86 (IQR 94.47–214.20); Me $_{\text{Se:Hg in Hg-U}}$: 80.46 (IQR 51.26–97.80); $p < 0.0001$ for both comparisons).

### 3.5. Assay of markers of the antioxidant effect

Median values of GPx1-RBC and GPx3-P activities as well as concentration of SeP-P among the chloralkali workers were presented in Table 1. Similar results for GPx1-RBC and GPx3-P were obtained for both groups. Concentration of SeP-P was higher in the control group than that in the Hg°–exposed workers Me $_{\text{control}}$: 7.64 µg/L (IQR 3.91–12.35 µg/L) vs. Me $_{\text{exposed}}$: 5.83 µg/L (IQR 3.10–13.39 µg/L) but the difference was statistically insignificant ($p = 0.2122$). However, when the workers were divided into two groups according to the low and high concentrations of Hg-B (using the median), concentration of SeP-P differ statistically (Me $_{\text{low Hg-B}}$: 4.40 µg/L (IQR 2.48–8.93 µg/L) vs. Me $_{\text{high Hg-B}}$: 6.64 µg/L (IQR 4.42–14.27 µg/L); $p = 0.0033$).

Determination of TAA-P measured as a total antioxidant activity in plasma showed significantly higher levels (8% higher) among the workers from the chemical plant compared with the controls (Me $_{\text{exposed}}$: 1.98 mmol/L (IQR 1.78–2.10 mmol/L) vs. Me $_{\text{control}}$: 1.84 mmol/L (IQR 1.62–1.99 mmol/L), $p = 0.0038$).

### 3.6. Gene expression

Comparison between genes expression in the peripheral blood leukocytes between the study groups is presented in Table 3. *SEPP1* gene expression was determined in 85% of all the samples from the occupationally exposed group and 79% from the control group. Compared to the control group, the *SEPP1* gene expression of the workers was not significantly different ($p = 0.1028$). However, when the workers were divided into two groups according to the low and high concentrations of Hg-B (using the median), expression of *SEPP1* differ statistically (Me $_{\text{low Hg-B}}$: 4.52 (IQR 4.03–5.13 vs. Me $_{\text{high Hg-B}}$: 4.95 (IQR 4.22–5.34); $p = 0.0358$).

A significant increase in the levels of mRNA encoding *GPX1* ($p = 0.0002$) and *PRDX1* ($p = 0.0001$) was shown among the Hg°–exposed workers when compared to the unexposed group. Median of gene expression amounted to 13.50 (IQR 13.16–13.79) for *GPX1* and to 11.65 (IQR 11.54–11.86) for *PRDX1* in the exposed group and to 13.24 (IQR 12.94–13.48) for *GPX1* and 11.50 (IQR 11.34–11.75) for *PRDX1* in the controls (Fig. 2).

Comparison TRXR1 gene expression between two examined groups did not differ statistically ($p = 0.1114$). But when the workers were divided into two groups according to the low and high concentrations of Hg-U (using the median), expression of TRXR1 differ statistically (Me

**Table 3**
Comparison between gene expressions in peripheral blood leukocytes between the study groups.

| | Chloralkali workers (n = 131) | | | Control group (n = 67) | | | p |
|---|---|---|---|---|---|---|---|
| | N | Median | IQR | N | Median | IQR | |
| *GPX1* | **130** | **13.50** | **(13.16–13.79)** | **66** | **13.24** | **(12.94–13.48)** | **0.0002** |
| *GPX3* | 130 | 4.86 | (4.44–5.27) | 65 | 4.70 | (4.22–5.24) | 0.1653 |
| *SEPP1* | 111 | 4.65 | (4.12–5.20) | 53 | 4.87 | (4.41–5.60) | 0.1028 |
| *PRDX1* | **130** | **11.65** | **(11.54–11.86)** | **66** | **11.50** | **(11.34–11.75)** | **0.0001** |
| *PRDX2* | 130 | 11.13 | (10.84–11.40) | 66 | 10.90 | (10.71–11.27) | 0.0850 |
| *TRXR1* | 130 | 5.85 | (5.54–6.21) | 66 | 6.20 | (4.96–6.73) | 0.1114 |
| *TRX1* | 130 | 10.52 | (10.22–10.78) | 66 | 10.58 | (10.20–10.92) | 0.3771 |

Statistically significant p-values are shown in bold. Mann-Whitney's test. IQR – The interquartile range.

$_{\text{low Hg-B}}$: 5.95 (IQR 5.64–6.27) vs. Me $_{\text{high Hg-B}}$: 5.71 (IQR 5.43–6.19); $p = 0.0237$).

Moreover, when the workers were divided into two groups according to the low and high concentrations of Se (using the median), expression of the examined genes did not differ statistically ($p > 0.05$).

### 3.7. Relationships between mercury level and other variables

We did not find significantly difference between smokers and non-smokers in Hg-U concentration in the occupationally exposed group (Me $_{\text{smokers}}$: 14.01 μg/g creat. (IQR 8.99–18.84 μg/g creat.) vs. Me $_{\text{non-smokers}}$: 10.35 μg/g creat. (IQR 6.66–21.04 μg/g creat.); $p = 0.1568$) and was of borderline significance in the control group (Me $_{\text{smokers}}$: 0.12 μg/g creat. (IQR 0.11–0.19 μg/g creat.) vs. Me $_{\text{non-smokers}}$: 0.17 μg/g creat. (IQR 0.14–0.25 μg/g creat.); $p = 0.0562$). In the control group, a person who smoked had a lower concentration of Hg-B ($p = 0.0450$).

In the control group, people with dental amalgam fillings had a higher Hg-U concentration (Me $_{\text{dental amalgam fillings}}$: 0.19 μg/g creat. (IQR 0.15–0.37 μg/g creat.) vs. Me $_{\text{no dental amalgam fillings}}$: 0.15 μg/g creat. (IQR 0.11–0.20 μg/g creat.); $p = 0.0107$) but Hg-B concentration was not altered (Me $_{\text{dental amalgam fillings}}$: 0.52 μg/L (IQR 0.31–0.66 μg /L) vs. Me $_{\text{no dental amalgam fillings}}$: 0.52 μg/L (IQR 0.37–0.84 μg/L); $p = 0.4993$). Moreover, the number of dental amalgam fillings in the teeth correlated positively with Hg-U (R = 0.54, $p = 0.0094$), but not with Hg-B (R = −0.03, $p = 0.8784$).

Hg-B as well as Hg-U seems not to be affect by participants' age (all absolute R values < 0.15 and all p values > 0.15). Correlation between BMI and Hg-B among the chloralkali workers was positive and at borderline significance (Table 4). A statistically significant, positive correlation was found between the biomarkers of exposure to Hg, meaning Hg-U and Hg-B in both groups – in the occupationally exposed to Hg° group (R = 0.57; $p < 0.0001$) and in the control group (R = 0.48; $p < 0.0001$). There was also a significant positive correlation between duration of Hg° exposure (in the workplace) and concentration of Hg-B (R = 0.32; $p = 0.0002$) and Hg-U (R = 0.25; $p = 0.0057$).

In the occupationally exposed group, concentration of Hg-U positively correlated with TAA-P (R = 0.23; $p = 0.0085$), but it correlated negatively with concentration of Se-U (R = −0.25; $p = 0.0047$) and Se-P, but in this case it was of borderline significance (R = −0.17; $p = 0.0529$).

Moreover, in the exposed group, concentrations of Hg-B and Hg-U were positively correlated with SeP-P concentration (R = 0.32; $p = 0.0002$, R = 0.20; $p = 0.0236$; respectively) as well as with its gene expression encoding SEPP1, but the correlation between Hg-U and *SEPP1* was of borderline significance (R = 0.25; $p = 0.0077$, R = 0.18; $p = 0.0575$; respectively). An inverse association was observed between Hg-U and *TRXR1* expression among the Hg°-exposed workers (R = −0.26; $p = 0.0026$). Expression of PRDX1 revealed a negative association with concentration of Hg-B and Hg-U, but both them were at the margin of statistical significance (R = −0.18; $p = 0.0454$, R = −0.17; $p = 0.0585$; respectively). In the control group there was not a significant relationship between Hg-B and PRDX1 expression but we found a significant positive correlation between Hg-B and PRDX2 (R = 0.30; $p = 0.0151$).

Further investigation of the previously observed correlation between *SEPP1, PRDX1*, *TRXR1* genes expression and biomarkers of occupational exposure to Hg° was conducted using the multivariate regression models. To examine the relationship between the selected genes expression and Hg-U concentration, several potential confounders, including: dental amalgam fillings, age, BMI, job seniority

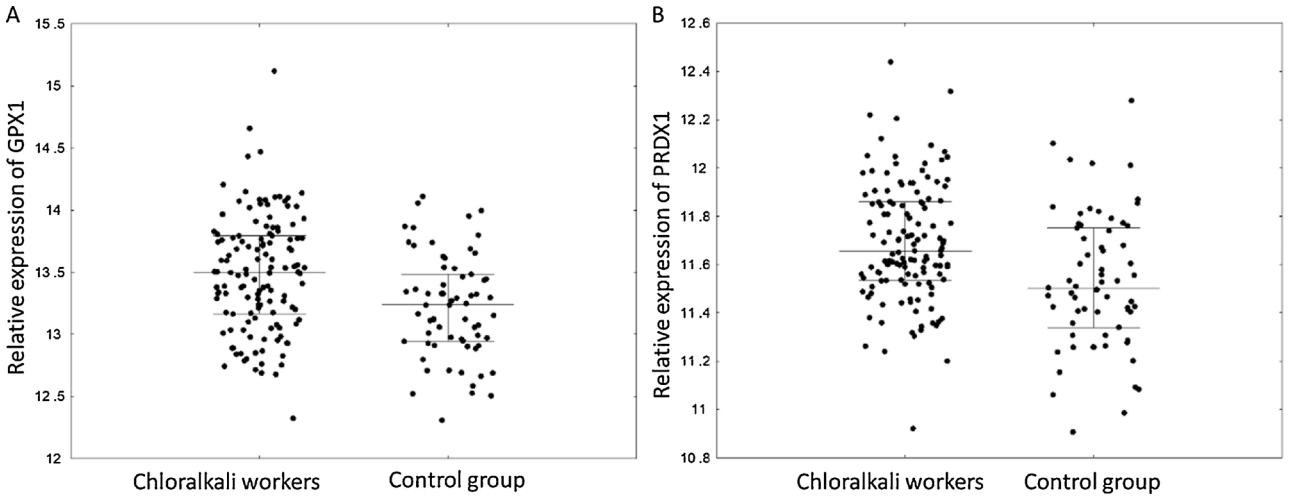



**Fig. 2.** Graphs of selected significant differences in expression of *GPX1* (left Figure, A) and *PRDX1* (right Figure, B) in people work in a chemical plant. Horizontal line represents median value and whiskers represent 25%–75% range.

**Table 4**
Associations between Hg-B and Hg-U and examined parameters.

| Parameters | Hg-B (μg/L) | | | | | | Hg-U (μg/g creat.) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chloralkali workers | | | Control group | | | Chloralkali workers | | | Control group | | |
| | N | R | p | N | R | p | N | R | p | N | R | p |
| Age (years) | 131 | −0.09 | 0.3241 | 67 | 0.09 | 0.4758 | 131 | −0.12 | 0.1805 | 67 | 0.03 | 0.7859 |
| BMI (kg/m$^2$) | 131 | 0.15 | 0.0835 | 67 | 0.00 | 0.9743 | 131 | −0.09 | 0.3270 | 67 | −0.04 | 0.7213 |
| Duration of exposure to Hg° (in the workplace) (years) | **125** | **0.32** | **0.0002** | | | NA | **125** | **0.25** | **0.0057** | | | NA |
| Duration of exposure to Hg° (in the plant) (years) | 131 | −0.09 | 0.3288 | | | NA | 131 | −0.10 | 0.2792 | | | NA |
| The number of dental amalgam fillings | 43 | −0.20 | 0.1930 | 22 | −0.03 | 0.8784 | 43 | 0.28 | 0.0726 | **22** | **0.54** | **0.0094** |
| Hg-B (μg/L) | | | | | | | **131** | **0.57** | **< 0.0001** | **67** | **0.48** | **< 0.0001** |
| Hg-U (μg/g creat.) | **131** | **0.57** | **< 0.0001** | **67** | **0.48** | **< 0.0001** | | | | | | |
| GPx3-P (U/mL) | 131 | −0.00 | 0.9767 | **67** | **0.24** | **0.0497** | 131 | −0.05 | 0.5333 | 67 | −0.02 | 0.8471 |
| GPx1-RBC (U/gHb) | 131 | −0.00 | 0.9735 | 67 | −0.04 | 0.7474 | 131 | −0.15 | 0.0921 | 67 | 0.01 | 0.9434 |
| SeP-P (μg/L) | **131** | **0.32** | **0.0002** | 67 | 0.09 | 0.4783 | **131** | **0.20** | **0.0236** | 67 | −0.02 | 0.8921 |
| TAA-P (mmol/L) | 131 | 0.03 | 0.7631 | 67 | −0.20 | 0.1066 | **131** | **0.23** | **0.0085** | 67 | 0.01 | 0.9571 |
| Se-P (μg/L) | 131 | −0.06 | 0.4924 | 66 | 0.19 | 0.1246 | **131** | **−0.17** | **0.0529** | 66 | 0.11 | 0.3890 |
| Se-U (μg/g creat.) | 131 | −0.07 | 0.4247 | 67 | 0.09 | 0.4452 | **131** | **−0.25** | **0.0047** | 67 | −0.02 | 0.8797 |
| Ln *GPX1* | 130 | 0.06 | 0.4808 | 66 | 0.02 | 0.8894 | 130 | 0.05 | 0.5847 | 66 | −0.03 | 0.7888 |
| Ln *GPX3* | 130 | −0.05 | 0.5794 | 65 | 0.10 | 0.4184 | 130 | −0.06 | 0.5088 | 65 | 0.15 | 0.2214 |
| Ln *SEPP1* | **111** | **0.25** | **0.0077** | 53 | 0.02 | 0.9015 | 111 | 0.18 | 0.0575 | 53 | −0.01 | 0.9233 |
| Ln *PRDX1* | **130** | **−0.18** | **0.0454** | 66 | 0.12 | 0.3271 | 130 | −0.17 | 0.0585 | 66 | 0.16 | 0.1938 |
| Ln *PRDX2* | 130 | 0.07 | 0.4193 | **66** | **0.30** | **0.0151** | 130 | 0.05 | 0.5694 | 66 | 0.19 | 0.1344 |
| Ln *TRXR1* | 130 | −0.06 | 0.4628 | 66 | −0.03 | 0.8203 | **130** | **−0.26** | **0.0026** | 66 | −0.07 | 0.5770 |
| Ln *TRX1* | 130 | −0.06 | 0.4690 | 66 | −0.00 | 0.9839 | 130 | −0.08 | 0.3891 | 66 | −0.01 | 0.9640 |

Value of R was calculated using Spearman's correlation coefficient. Statistically significant p-values were bolded.

**Table 5**
Multivariate modeling for the analysis of relationships between selected genes expression and Hg° exposure marker and confounding factors.

| Variable | Ln *TRXR1* | | Ln *SEPP1* | | Ln *PRDX1* | |
|---|---|---|---|---|---|---|
| | R2 corrected = 0.07, p = 0.0186 | | R2 corrected = 0.01, p = 0.2910 | | R2 corrected = −0.04, p = 0.9382 | |
| | β | 95%CI | β | 95%CI | β | 95%CI |
| Hg-U (μg/g creat.) | **−0.30** | **−0.48 to −0.12** | 0.08 | −0.12 to 0.28 | −0.11 | −0.30 to 0.07 |
| Dental amalgam fillings (1 = Yes, 2 = No) | −0.07 | −0.24 to 0.11 | 0.02 | −0.18 to 0.22 | −0.03 | −0.21 to 0.15 |
| Age (years) | −0.08 | −0.29 to 0.12 | −0.08 | −0.30 to 0.14 | −0.07 | −0.29 to 0.14 |
| BMI (kg/m$^2$) | 0.14 | −0.04 to 0.32 | **0.22** | **0.02 to 0.43** | 0.03 | −0.16 to 0.22 |
| Smoking (1 = Yes, 2 = No) | −0.12 | −0.30 to 0.05 | −0.07 | −0.27 to 0.13 | −0.02 | −0.21 to 0.16 |
| Duration of exposure to Hg° (in the plant) (years) | 0.15 | −0.05 to 0.35 | 0.11 | −0.11 to 0.33 | 0.04 | −0.17 to 0.25 |

β – beta coefficient. Statistically significant p-values were bolded.

time and smoking habits were taken into consideration (Table 5). In the multivariate model, we confirmed that Hg-U concentration was inversely correlated with *TRXR1 expression* (β = −0.30, 95% CI = −0.48 to −0.12; $p$ = 0.0186). We found no relationship between concentration of Hg-U and *PRDX1* (β = −0.11, 95% CI = −0.30 to 0.07; $p$ = 0.9382) and *SEPP1* (β = 0.08, 95% CI = −0.12 to 0.28; $p$ = 0.2910).

## 4. Discussion and conclusion

Although research had an observational nature, this paper is the first comprehensive assessment of the impact of occupational exposure of workers to Hg° at both the mRNA and selenoprotein levels, with investigation of fish intake obtained by means of a questionnaire.

Some authors have suggested that the Hg–Se interaction is most efficient when Se and Hg are administered at the same time in an equimolar ratio [17,18]. The above studies were in resemblance to the results of Peterson et al. [19] and Li et al. [20], that have shown that toxic effects of Hg are counteracted by Se, but only when Se:Hg molar ratios approach equaled or exceed 1. Similar equimolar ratio has been found by Kosta et al. [21] in kidney, liver and muscle tissues of the miners. Chen et al. [11] found that the molar ratio of Se:Hg close to 1 in urine of the miners was 0.7 ± 0.6. Such a relationship was also confirmed in our work, where the molar ratio of Se:Hg slightly exceeded 1, but only in the urine.

As expected, the occupationally exposed group in our study had significantly higher ($p$ < 0.001) Hg concentrations in biological fluids when compared to the control group, which suggests that the individuals were occupationally exposed to Hg°, which is in accordance with the results of Barregård et al. [8], Bulat et al. [9], Samir and Aref, [13]. Additionally, the Se status determined in our study did not differ from such statuses obtained in the studies performed, on the populations of Central Europe [22,23] and concentrations of Se in the individuals without Hg° exposure are within the range of mean values for Polish population [24,25].

As we measured total Hg and Se concentration (without speciation), it is assumed that a part of concertation of Hg and Se might have come from a diet. That is why, we carried out extensive questionnaire research concerning eating habits in the two examined groups. The analysis of these results showed no significant differences in terms of the intake of fish and meat products, which confirms a similar diet of both the examined groups. Moreover, the workers did not indicate excessive fish consumption and thus, we can assume that they were not exposed to the organic form of mercury. Therefore, the analysis of the impact of MeHg in blood, linked to fish consumption, is negligible and can be omitted. In the case of exposure to a higher concentration of Hg° or more frequent changes in exposure conditions, analysis of Hg-B seems to provide better results with respect to the current exposure

assessment. The analysis of the distribution of food consumption frequency with regard to fish and meat products category is presented in Fig. 1.

Assessment of the relationship between Hg and Se in our study indicated that urinary Hg concentration was significantly inversely correlated with Se-U as well as Se-P only in the occupationally exposed group.

As we mentioned, because the concentration of Se and Hg in the blood are biomarker of short-term exposure, the concentration of Se and Hg in the urine can more accurately reflect their metabolism and excretion after long-term exposure to Hg°. This downhill relationship observed in the workers may suggest that retention of Se increased in the Hg°-exposed workers group. The study of Barregård et al. [8] showed that Se-U was negatively correlated with Hg-P in the occupationally exposed (n = 37) group compared to the controls (n = 39), which may indicate the interference of Hg with Se but only in a recent exposure. Higher exposure levels to Hg in chloralkali workers decrease Se-U excretion, which was confirmed also in the study of Hongo et al. [26], but these authors suggested that concentration of Se-U reflected dietary Se intake and the variance of excretion Se-U might be accidental.

Despite decades of Hg research, data on the occurrence of correlation between exposure to inhalation of elemental mercury and its metabolism and levels of selenoproteins in the mRNA as well as proteins among workers who are occupationally exposed to Hg° are still limited [6,9,11–13]. Our study supplements current knowledge in this field. Environmental exposure to Hg appears to alter gene expression profiles, which directly corresponds to a change in protein levels. As a consequence, Hg may alter enzymatic activity of proteins.

Studies of Chen et al. [11] showed that Hg exposure causes stress reactions via enzyme activation. In their study an increase in activity of GPx in serum of Guizhou (China) miners (n = 37) in comparison with control group (84.6 ± 12.1 U/ml/min. vs. 75.0 ± 14.1 U/ml/min.) ($p < 0.05$) was observed. In addition, the increase in the activity of antioxidant enzyme GPx and concentration of SeP was also accompanied by a decreased production of ROS. Additionally, those authors showed that concentration of Hg in SeP was significantly higher among Hg-exposed people than in their control group and amounted to 19.4 ± 9.2 μg /L vs. 0.15 ± 0.12 μg/L ($p < 0.05$) and the molar ratio Se:Hg in SeP amounted to 7.8 ± 3.1 vs. 535 ± 216 ($p < 0.05$), respectively. Therefore, we can conclude, that a high concentration of Se may induce an increased synthesis of SeP in the case of which the rest of selenocysteine, being a component of SeP acts like a trap for Hg keeping it in circulation and preventing its accumulation in organs.

We assessed the positive correlation between selenoprotein P levels (at both the mRNA and protein levels) and Hg in both media (Hg-B and Hg-U). The results may indicate that detoxification processes have been activated in workers exposed to Hg°. Thus, the presence Hg-Se-protein complex in the body may slightly reduce the pool of bioavailable Se in the body, and reduce the concentration of Se-U. Additionally, a moderate uphill relationship between Hg-B and Hg-U concentrations in both the studied groups and between Se-P and Se-U concentrations only in the Hg°-exposed group (unpublished data) may reflect an altered metabolism and excretion of these chemical elements in the exposed group. The interference of Hg with the metabolic pathways of Se has been confirmed in numerous studies [8,27,28].

Chen et al. [11] showed that miners who were occupationally exposed to Hg presented an increased expression of genes encoding *SEPP1* and *GPX*. This increase was accompanied by an increased Se concentration in serum. Moreover, a strong positive correlation between concentrations of Se and Hg (R = 0.625, $p < 0.001$) was observed in the urine, but not in the serum. Similarly, our study, indicated a statistically higher concentration of Se-P, TAA-P as well as expression of *GPX1* among the subjects occupationally exposed to Hg°, which may indicate an induction of antioxidant defense mechanisms. However, contrary to Chen et al. [11] we found a negative correlation between urinary Hg and Se concentrations (Se-U and Se-P). Kobal et al. [6] in a similar study concerning the impact of a long-term occupational Hg exposure on erythrocyte glutathione level and activities of antioxidative enzymes did not find any differences ($p < 0.05$) between their miners (n = 28) and controls (n = 41) in terms of the mean GPx activity, mean concentration of total GSH, oxidized disulphide glutathione (GSSG) levels, and GSH/GSSG ratio, although the mean concentration of reduced GSH was significantly higher ($p < 0.05$) in the miners (13.64 ± 3.71 mmol/g Hb) in comparison with the control group (11.68 ± 2.66 mmol/g Hb).

Opposite results were shown in the study by Bulat et al. [9] in workers (n = 42) occupationally exposed to Hg in a chloralkali plant. The GPx activity in erythrocytes of the workers (GPx: 9.05 ± 7.52 IU/g Hb) was significantly lower ($p < 0.001$) than in the control group (GPx: 15.54 ± 4.85 IU/g Hb). Determined decreased activity of the antioxidant enzyme was accompanied by erythrocyte Se level. It seems right to believe, that Se-dependent enzyme is directly influenced by Se concentration in the body. Exposure to Hg decreased activity of GPx in erythrocytes and potential detoxification mechanism in the people exposed to Hg. The above mentioned study is consistent with Samir and Aref, [13], who showed the impact of occupational exposure (dental personnel, n = 32) to elemental Hg on some antioxidative enzymes. Compared to their control group, the GPx activity in blood of the workers was significantly decreased ($p < 0.001$) [13]. Moreover, authors noted, that a significant negative correlation was found between Hg-U and GPx activity in blood of the occupationally exposed group in comparison with the control group ($r = -0.668$, $p < 0.001$). Additionally, a comparison of the duration of work among dental professionals revealed a positive significant correlation with Hg-U and Hg-B ($r = 0.40$, $p < 0.05$; $r = 0.436$ $p < 0.05$ respectively), which is similar to our study, and a negative correlation with GPx ($r = -0.656$, $p < 0.001$). These results may indicate that exposure to Hg decreased activity of GPx in erythrocytes and potential detoxification mechanism for renal dysfunction in people who were exposed to Hg, which was revealed through the decreased activities of antioxidant enzymes.

Although we were unable to show any statistically significant differences in outcomes between the exposed subjects and the controls in the activity of both GPxs in our study, (being in agreement with outcomes of Kobal et al. [6]; Barregård et al. [8] or Molin et al. [29]), the median value of the *GPX1* and *PRDX1* expression in the occupationally exposed group was significantly higher, which is indicated by a dispersion of a data set on Fig. 2.

A number of findings indicate that mercury inhibits protein components of the thioredoxin system both in vitro [30,31,33] and in vivo [32,33]. TrxR is more sensitive than other selenoproteins, because of its highly nucleophylic structure. In our study, we found an inverse correlation between Hg-U and *TRXR1* expression in the Hg°-exposed workers. The multivariate analysis that included several covariates confirmed a weak negative correlation between Hg-U and *TRXR1* expression. Inhibition of the thioredoxin system by Hg treatment was confirmed by animal model studies [32,33]. It has been proven that Hg inhibited the activity of selenoenzyme TrxR in juvenile zebra-seabreams by 50% [32] and in experiments with rats, mercuric chloride ($HgCl_2$) inhibited TrxR (in concentration 50 nM) with $IC_{50}$ value of 7.2 nM and MeHg with $IC_{50}$ 19.7 nM) [30]. Moreover, the mass spectra analysis has shown the very strong divalent mercury complexes involving active site of TrxR and structural disulfides in contrast to MeHg [30]. It is connected with different chemical properties of the 2 forms. Although both of them bound to Trx1, only $HgCl_2$ generated dimers. As a consequence, inorganic Hg had higher inhibition potency than the organic form of Hg. The most appealing explanation is that, the monovalent MeHg is less electrophilic.

The inhibitory effect of Hg on TrxR in the case of in vitro assays was observed very quickly, i.e. - in 5 min. Inhibition was dose-dependent, but not time-dependent. Moreover, authors showed that Hg-induced inhibition was selective in the direction of the thioredoxin system [30].

This assumption could be confirmed by our results, where we indicated that genes of *TRXR1* and *PRDX1* are particularly susceptible to inhibition by mercury. We suggest that the extraordinary potency of Hg compounds to bind to the selenol-thiol in the active site of TrxR is a major molecular mechanism of Hg toxicity. Inhibition of the thioredoxin system, which maintains intracellular redox homeostasis under physiological conditions may in consequence restrict oxidative stress-induced Hg response pathways. In this statement, a pro-oxidant role of Hg may be revealed and exposure to Hg may exert an opposite effect on the selenoproteins, depending on the specific selenoprotein.

On the other hand, basing on the results obtained in our project, we can suggest that the mechanism of this type of inhibition may result from the depletion of Se level in the body (due to the formation of Se-Hg complexes with SeP). Thus, a negatively correlation between Hg and genes expression of *TRXR1* and *PRDX1* may result from this mechanism. However, no further data concerning the impact of occupational exposure to Hg° on the thioredoxin system suggest that, and so this type of conclusions should be interpreted with caution and require additional research.

In conclusion, we observed that exposure to Hg° affects Se status which is accompanied by a disturbed expression and activity of selected selenoproteins involved in the antioxidant defense system. The mRNA level for *GPX1, PRXD1* were markedly but significantly higher in the workers compared to the control group. Moreover, the positive relationship between biomarkers of Hg° exposure and selenoprotein P at both the mRNA and protein levels, and between levels of Hg-U and TAA-P may point to that exposure to Hg° is associated with induction of a compensatory upregulation of the antioxidant selenoproteins and hence with activation of antioxidant response pathway in humans.

In our study, we also found an inverse correlation between Hg-U and *TRXR1* expression in the Hg°-exposed workers, which may indicate a selective inhibition of thioredoxin reductase by mercury.

Author contributions

RK drafted the manuscript and constructed the database. RK, ER, EW, EJ, JG, JB and MS performed analysis. BM contributed to statistical analyses, LK performed food frequency questionnaire, WW contributed to the study design. All co-authors revisions of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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