Oxidative Stress and Arsenic Exposure among Copper Smelters

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ASA, AS, LR and HT, designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AS, HT designed the patient files and managed the literature searches. Authors AS, HT, managed the analyses of the study, and wrote the discussion and recommendations of this study. Author AES revised the discussion and recommendations. All authors read and approved the final manuscript.

ABSTRACT

Copper is widely used in industry. It has been associated with several health hazards among exposed workers.

Aim: to measure the indicators of oxidative stress as malondialdehyde level and superoxide dismutase enzyme activity and their association with copper and arsenic levels among copper smelter workers.

Subjective and Methods: This study was conducted on forty workers in a secondary copper smelting factory, who were occupationally exposed to copper. They were compared with forty non-exposed individuals. Full history, clinical examinations were done. Serum copper, serum arsenic, urinary arsenic, malondialdehyde and superoxide dismutase were measured. Environmental measurements of copper and arsenic dusts were carried out at different workplace areas.

Results: Environmental measurements in the workplace were within the normal permissible limits in Egypt. Statistically significant differences were found between exposed and control as regards the prevalence of the respiratory and neurological...
symptoms. Compared to the control group, serum copper, serum arsenic, urinary arsenic and malondialdehyde blood levels were significantly higher among the exposed worker ($P<0.01$). Each one was positively correlated with the duration of employment. Superoxide dismutase activities in blood were significantly decreased and negatively correlated with the duration of employment.

**Conclusion:** The disruption of hemostasis induced by oxidative stress may promote the development of health hazards with continued occupational exposure to copper fumes. **Recommendation:** Blood levels of malondialdehyde and superoxide dismutase enzyme activity can be used as indicators of oxidative stress among exposed workers.

**Keywords:** Copper smelters; arsenic; malondialdehyde (MDA); superoxide dismutase (SOD); oxidative stress.

### 1. INTRODUCTION

Mining and smelting of heavy metals can be traced back thousands of years ago. Copper occasionally occurs native as elemental copper in ores and minerals. The most important copper ores are the sulfides, oxides and carbonates. From these, copper is obtained by smelting, leaching, and electrolysis [1]. Emissions from copper smelter are principally particulate matter and sulfur oxides. Chronic exposure to inorganic arsenic involves a biotransformation process that led to the main excretion of organic methylated metabolites, such as monomethyl arsionic acid (MMA) and dimethylarsinic acid (DMA), as well as the parental inorganic species [2]. Arsenic is a naturally occurring element widely distributed through the Earth’s crust. It is a toxic and volatile element that has little commercial use. This is causing some concern to copper smelters. Arsenic materials produced as a by-product during the smelting process [3]. At the molecular level, Physic-pathological effects related to arsenic toxicity appear to involve different mechanisms and intracellular targets. Oxidative stress is among the most documented mechanisms of arsenic toxicity and carcinogenicity. It is the result of an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system, e.g. Superoxide dismutase (SOD) [4]. Radical oxygen species (ROS) production of arsenic may result in an attack, not only against antioxidant defenses and DNA, but also against membrane phospholipids, which are very sensitive to oxidation, producing peroxyl radicals and then malondialdehyde (MDA) [5].

Both short and long term exposure to arsenic can cause several health problems, including noxious effects on cardiovascular [6], lung [7, 8], skin [9] and neurological systems [10]. Mental status like stress, emotion and disease conditions are also responsible for the formation of free radicals. Additionally, the hormones that mediate the stress reaction in the body like cortisol and catecholamine themselves degenerate into destructive free radicals [10]. In regard to toxicity, the International Agency for Research on Cancer (IARC) defines arsenic as a group I known human carcinogen that causes a wide range of other noncancer effects, leaving no system free from potential harm [11]. Chronic exposure of humans to high concentrations of arsenic in drinking water is associated with skin lesions, peripheral vascular disease, hypertension, Blackfoot disease, and high risk of cancers. However, in the field of occupational medicine, no sufficient data have been available on the relationship between oxidative stress and chronic exposure to arsenic among copper smelters. Experimental studies [12-15], found an increase in lipid oxidation markers as MDA and decrease in the activity of antioxidant enzymes, e.g. (SOD) among animals exposed to high levels of metals like Cu and as. This study was designed to evaluate the indicators of
oxidative stress and their association with the level of copper and arsenic levels among copper smelter workers. To execute this task, the researchers designed the objectives, as follows: 1-To measure copper and arsenic in the environment of a copper smelter factory. 2- To determine the prevalence of some health hazards among exposed workers. 3- To measure serum copper, serum arsenic and urinary arsenic. 4-To assess the level of MDA (malondialdehyde) lipid peroxidation product and SOD (superoxide dismutase) enzyme activity in blood as indicators of oxidative stress among exposed workers.

2. SUBJECTS AND METHODS

2.1 Study Population

This study was carried out at the factory in Helwan, Cairo, Egypt, that contains many sectors, one of these sectors is a secondary copper smelter during the period from September 2012 to November 2012. A case control study was conducted on two groups: an exposed and a control group. The first group included forty male (40) workers on the production line in the secondary copper smelter. The total number of working population who engaged in the process of secondary copper smelting were one hundred (100) workers. Simple randomization enrolled fifty (50) workers, however, only forty five (45) workers from the 3 shifts within the factory who accepted to participate in this survey, and five (5) workers were excluded from the study according to exclusion criteria. The inclusion and exclusion criteria were as follows: Inclusion criteria: included those workers exposed to copper more than two years. Exclusion criteria included those subjects diagnosed as diabetic and/or receiving treatment for diabetes, hypertensive subjects and/or receiving antihypertensive treatment, having any chronic illness as cardiovascular diseases or receiving antioxidant drugs or any treatment during the last 6 months were excluded. The control group included forty male workers in administering departments of the same company, who have never been occupationally exposed to copper. Both groups were matched for age, sex, socioeconomic status and smoking habit. For the controls; the same criteria above were used except for being in contact with copper process.

2.2 Methods

The study was first approved by the ethical committee of the Department of Occupational and Environmental Medicine, Cairo University, Egypt. Prior to this study, a written consent to share the study and an approval to give blood samples from each individual were obtained after explaining to them the aim and the importance of the study. During the study, the ethical guidelines of good clinical practices (GCPs) have been explained. Strict confidentiality was observed throughout sample collection, coding, testing, and recording of the results. The studied groups were subjected to a specially designed detailed questionnaire including: socio-demographic data including age, residence, marital status, and smoking habits, present, past and family history. Occupational history included: current job and its nature, previous jobs, duration of employment in years; using protective equipment or not. Health complaints: onset, duration and relation to work. The survey was conducted using face-to-face interviews in the local language of the country.

2.3 Laboratory Investigations

From each subject 10 cc of venous blood was taken through a vein puncture using a dry plastic disposable syringe under complete aseptic condition. Three milliliters of blood were
taken into a clean tube containing dipotassium ethylene diamine tetra acetate (EDTA) as an anticoagulant for determination of MDA and SOD levels in blood. The remaining blood was kept in a separate tube and allowed to clot, then centrifuged for separation of the serum. All samples were transported to the Clinical Chemistry laboratory in the clinical pathology Department, Faculty of Medicine, Cairo University (Cairo, Egypt), where they were analyzed.

2.4 Determination of Arsenic and Creatinine Levels in Urine

During the physical examinations, random urine specimens were collected in arsenic-free containers, and sent to the Clinical Chemistry Department, Faculty of Medicine, Cairo University (Cairo, Egypt), where they were analyzed for Arsenic and creatinine levels in urine. Urinary arsenic was determined by graphite furnace atomic absorption spectrometry. Arsenic concentrations were adjusted using creatinine concentrations and expressed as arsenic/ creatinine ratio (microg/g creatinine) to correct for variable water excretion rates at the time of spot urine specimen collection [14]. Creatinine was measured by an automated colorimetric method based on a modified Jaffe reaction on Hitachi 917 auto-analyzer using a kit purchased from Roche (Roche Diagnostics GmbH, D-68298 Mannheim). All the methods used were certified according to guidelines set forth in the Clinical Laboratory Improvement Amendment (CLIA) [15].

2.5 Determination of Serum Copper, Serum Arsenic and Urinary Arsenic

Serum and urinary arsenic were measured by hydride generation atomic absorption spectrophotometer with zemman background (Thermo elemental M-6 Type). Serum copper level was measured by flame atomic absorption spectrophotometer with zemman background (Thermo elemental M-6 Type). The samples for arsenic and copper were prepared by dilution of 0.5 ml of blood with 2 ml deionized water and then centrifuged to obtain hemolysate. External Calibrators for arsenic and copper were prepared by serial dilution of parent stock which contains 1000 µl /ml using the diluents (deionized water). For the reading metals concentration of both samples and standard (calibrator), it was important to choose proper wave length, lamp current band pass optimization for each metal. By plotting standard curve, the reading of the absorbance of the sample and calibrator was plotted on semi log curve; the concentration of each metal in samples was interpreted from the standard curve [16].

2.6 Determination of MDA (Malondialdehyde) Level

The samples were analyzed for malondialdehyde by the thiobarbituric acid (TBA) reaction (Daichi Pure Chemical Co. Ltd. Tokyo), with separation of MDA (TBA) adduct, using tetraethoxypropane (TEP) (Toyoko Kasei Co. Ltd. Tokyo) as the standard. Two ml sodium sulfate, 2.5 ml of 20% thiochloroacetic acid (TCA) and 1 ml of 0.67% (TBA) are added to 0.5 ml of blood. The coupling of lipid peroxide with TBA was carried out by heating in a boiling water bath for 30 min. The resulting chromgen was extracted with 4 ml of n-butyl alcohol and the absorbance of the organic phase is determined at wavelength 530 nm. Specific colorimetric method for the determination of lipid peroxide was used [17].

2.7 Determination of Superoxide Dismutase Activity (SOD) Blood Level

SOD Assay Kit-WST allows very convenient SOD assaying by utilizing Dojindo’s highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl) -3-(4-nitrophenyl) -5-(2,4-
disulfophenyl) -2H-tetrazoliummonosodium salt) that produces a water-soluble formal dye upon reduction with a superoxide anion. The rate of the reduction with O2 is linearly related to the xanthine oxidase (XO) activity and is inhibited by SOD. Therefore, the 50% inhibition activity of SOD or SOD-like materials can be determined by a calorimetric method [18].

2.8 Environmental Assessment

Environmental monitoring was carried out for air concentrations of copper and arsenic (workplace air sampling). A preliminary visit was done to inspect workplace for proper environmental assessment. The factory is composed of 2 compartments; administrative and production. The production unit consists of: (1) Melting of primary copper ingots and copper scraps in a large furnace [furnace (1) added it to zinc as hardener. It makes them more malleable and strong. Then the molten metal was poured through channels to another smaller treatment furnace while the slag was removed. (2) In the treatment furnace [furnace (2), nitrogen under pressure was passed through the molten metal with temperature (up to 850°C) to remove any air bubbles and for proper mixing of the metal with other additive. In the melting and pouring sections of the foundry, quantities of visible fumes were evolved with the hazards of exposure to copper oxide, other oxide of metals like arsenic, lead and zinc. (3) Cutting and the punching section, where the copper bars were cut to the desired length using electrical saw. Welding process occurs by the use of electrical source generated by certain generator during this process copper molds are welded using argon or oxyacetylene welding, resulting in emission of copper fumes, dust and particles.

Measurements were taken during shift time from three different places in copper production unit. Three measurements were taken from each place and the mean values are calculated. Samples of indoor air were collected in the production units by active sampling on 8× 110 mm² adsorbent tubes containing activated charcoal at a flow rate of 200 ml/min, using an air sampling pump with electronic flow control. The flow of the pump was calibrated using a mini-BUCK Calibrator M-30 Electronic Primary Gas Flow Standard, United States. After 4 h, the sampling was stopped by placing caps on both ends of the tubes. The tubes were covered with aluminum foil and stored at 4°C until analysis. Atomic absorption spectrophotometer, with appropriate hydrogen burner head or quartz tube furnace, and arsenic hollow cathode lamp or EDL and arsine generation system for arsenic and Atomic absorption spectrophotometer, with an air-acetylene burner head and copper hollow cathode lamp and two-stage regulators for air and acetylene for copper. Using the measured absorbance, calculate the corresponding concentrations (µg/ml) of copper in the sample, C_s, and average media, blank, C_b, from the calibration graph. Using the solution volumes (ml) of the sample, V_s and media blanks, V_b, the concentration, C (mg/m 3), of arsenic and copper in the air volume sampled, V (L) was calculated:

\[ C = \frac{C_s V_s - C_b V_b}{V} \]  [19,20].

Measurements were done in the Reference Laboratory – Environmental research Division - Air pollution Department - National Research Center, Cairo, Egypt. The measurements were compared to the maximum allowable limits according to Egyptian Environmental Law 4 for the year 1994 [21], Table 1.

2.9 Statistical Analysis

Data obtained from the study were coded and entered using the statistical package SPSS version 16. The mean values, standard deviations and ranges were estimated for quantitative variables; Qualitative data were represented as frequencies and percentages. Comparisons between exposed and control groups were carried out using Chi square and
the independent sample t test for quantitative variables which were normally distributed variables. Nonparametric Kruskal-Wallis for quantitative variables which were not normally distributed. The correlations between individual variables were calculated using Pearson correlation coefficient. p values <0.05 was considered statistically significant and P values >0.05 was not considered statistically significant.

3. RESULTS

The exposed group consisted of 40 male workers occupationally exposed to arsenic in the secondary copper smelter; their median age was 47.7±9.8 years, with employment duration of 25.9±9.5 years. The control group included 40 male workers. Their mean age was 48.0±8.1 years. There was no statistically significant difference between exposed and control groups as regards the age (p =0. 88). The frequency of smokers among exposed workers and control group were 20 (50%) and 21 (52.5%) respectively. Mean smoking index (no. Of cig. /Day × duration of smoking per year) among exposed workers and control group were 251.4±75.9; 248.0±63.4 respectively and there was no statistically significant difference (p =0. 83). Table 1 showed the measured air levels of copper and arsenic dust at different workplace areas. Measured Arsenic concentrations in the air of the melting furnace [furnace (1)] ranged from 0.0075 to 0.009 mg/m³; mean 0.008±0.00086, while copper concentrations ranged from 0.789 to 1.038 mg/m³; mean 0.805±0.225. In treatment furnace, furnace (2) arsenic concentrations ranged from 0.003 to 0.009 mg/m³, mean 0.006±0.0003, while copper concentrations ranged from 0.768 to 1.293 mg/m³; mean 0.989±0.272. In the cutting and the punching section, arsenic concentrations ranged from 0.0015 to 0.0045 mg/m³; mean 0.003±0.0015 while copper concentrations ranged from 0.420 to 0786 mg/m³; mean 0.569±0.192.

Table 1. Air levels of copper and arsenic dust at different workplace areas

<table>
<thead>
<tr>
<th>Workplace Area</th>
<th>Copper dust mg/m³ Mean±SD</th>
<th>Arsenic dust mg/m³ Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>The melting furnace 1</td>
<td>0.805±0.225</td>
<td>0.008±0.0008</td>
</tr>
<tr>
<td>The melting furnace 2</td>
<td>0.989±0.272</td>
<td>0.006±0.0003</td>
</tr>
<tr>
<td>The cutting and the punching section</td>
<td>0.569±0.192</td>
<td>0.003±0.0015</td>
</tr>
<tr>
<td>MAC*</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>ACGIH (TLV- TWA) **</td>
<td>1</td>
<td>0.01</td>
</tr>
</tbody>
</table>


In our study, we asked about using personal protective equipment (PPE) during work shift. We found that 14 (35%) of the exposed workers were using PPE; 10 (25%) were using gloves, followed by the masks 4 (10%).

All encountered health complaints (symptoms) have exposure relationships that emphasized by detailed history taking. Table 2 showed that the prevalence of dyspnea, cough, expectoration, chest pain, pain and peripheral paresthesias (numbness), headache, muscular fatigue and low back pain were statistically significant higher among the exposed workers than among the controls.
Table 2. The prevalence of symptoms among studied groups

<table>
<thead>
<tr>
<th></th>
<th>Exposed N=40</th>
<th>Control N=40</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N %</td>
<td>N %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>17 42.5</td>
<td>7 17.5</td>
<td>5.952</td>
<td>0.027</td>
</tr>
<tr>
<td>Dry cough</td>
<td>32 70</td>
<td>12 30</td>
<td>20.202</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Productive cough</td>
<td>23 57.5</td>
<td>10 25</td>
<td>8.717</td>
<td>0.006</td>
</tr>
<tr>
<td>Chest Pain</td>
<td>22 58</td>
<td>2 5</td>
<td>16.157</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pain and peripheral</td>
<td>18 45</td>
<td>6 15</td>
<td>8.571</td>
<td>0.007</td>
</tr>
<tr>
<td>Paresthesia(numbness)</td>
<td>18 45</td>
<td>6 15</td>
<td>8.571</td>
<td>0.007</td>
</tr>
<tr>
<td>Headache</td>
<td>24 60</td>
<td>10 25</td>
<td>10.026</td>
<td>0.013</td>
</tr>
<tr>
<td>Muscular fatigue</td>
<td>17 42.5</td>
<td>6 15</td>
<td>7.384</td>
<td>0.026</td>
</tr>
<tr>
<td>Low back pain</td>
<td>29 72.5</td>
<td>15 37.5</td>
<td>9.899</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Estimation of total mean values of serum copper, serum arsenic, urinary arsenic, superoxide dismutase enzyme activity (SOD) and malondialdehyde (MDA) levels in blood, showed that there were statistically significant higher levels among the exposed group compared to their controls as shown in Table 3.

According to Hassan et al. [22] values of MDA and SOD activity of the control group were considered.

Table 3. Mean±SD of serum copper, serum arsenic, urinary arsenic, superoxide dismutase (SOD) enzyme activity and Malondialdhyde (MDA) blood levels among studied groups

<table>
<thead>
<tr>
<th></th>
<th>Exposed (N=40)</th>
<th>Control (N=40)</th>
<th>t test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum copper (µg/dl)</td>
<td>148.4±15.6</td>
<td>90.7±9.7</td>
<td>59.324</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum arsenic (µg/l)</td>
<td>2.6±1.2</td>
<td>0.08±0.1</td>
<td>59.307</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary arsenic (µg/gcreat.)</td>
<td>38.7±14.2</td>
<td>3.7±9.7</td>
<td>53.778</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (u/ml)</td>
<td>185.5±19.8</td>
<td>232.3±10.4</td>
<td>57.063</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malondialdhyde (MDA) (nmol/ml)</td>
<td>5.6±1.9</td>
<td>1.4±0.6</td>
<td>58.032</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4 showed that the mean values of serum copper, serum arsenic, urinary arsenic, superoxide dismutase enzyme activity and malondialdehyde blood levels were statistically significant higher among smokers of exposed group than the control group, (p<0.001). Also Table 4 showed statistically significant differences between nonsmokers of exposed and control groups as regards serum copper, serum arsenic, urinary arsenic, superoxide dismutase enzyme activity and malondialdehyde blood levels, p<(0.001). Other comparisons between smokers and nonsmokers among exposed and control group are shown in Table 4.
Table 4. Mean±SD of serum copper, serum arsenic, urinary arsenic, superoxide dismutase enzyme activity and malondialdehyde blood levels among exposed and control group by different smoking habits

<table>
<thead>
<tr>
<th></th>
<th>Exposed N=40</th>
<th></th>
<th></th>
<th>Control N=40</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers N=21</td>
<td>Nonsmokers N=19</td>
<td>Smokers N=21</td>
<td>Nonsmokers N=19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum copper (µg/dl)</td>
<td>155.0±13.8†</td>
<td>141.1±14.3†</td>
<td>93.3±10.6</td>
<td>88.2±8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum arsenic (µg/l)</td>
<td>3.1±1.1††</td>
<td>2.0±1.1††</td>
<td>0.136±0.1*</td>
<td>0.02±1.1††</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary arsenic (µg/g creat.)</td>
<td>44.8±16.2††</td>
<td>31.9±7.2††</td>
<td>5.6±13.6*</td>
<td>1.8±1.3**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (u/ml)</td>
<td>193.6±18.9*</td>
<td>178.1±17.9††</td>
<td>233.1±8.5 †</td>
<td>231.5±12.1**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde (MDA) (nmol/ml)</td>
<td>4.7±1.7††</td>
<td>4.7±1.7††</td>
<td>1.7±0.61‡</td>
<td>1.1±0.3‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: p<0.001 (comparison between smokers of the exposed group and controls)
**: p<0.001 (comparison between nonsmokers of the exposed group and controls)
†: p<0.05 (comparison between smokers and nonsmokers among exposed group)
‡: p<0.001 (comparison between smokers and nonsmokers among the control group).

Correlations between different variables were shown in Table 5. Statistically significant positive correlations were found between Malondialdehyde (MDA) and each of; age, duration of employment, serum copper, serum arsenic and urinary arsenic, Table 5. Superoxide dismutase enzyme activity in blood was inversely correlated with each of age, duration of employment, serum copper, serum arsenic and urinary arsenic as shown in Table 5.

Table 5. Correlation coefficient between different variables and oxidative stress markers

<table>
<thead>
<tr>
<th></th>
<th>Serum Copper (µg/dl)</th>
<th>Serum arsenic (µg/l)</th>
<th>Urinary arsenic (µg/g creat.)</th>
<th>Superoxide dismutase (SOD) (u/ml)</th>
<th>Malondialdehyde (MDA) (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>r 0.171</td>
<td>0.162</td>
<td>0.693</td>
<td>-0.617</td>
<td>0.760</td>
</tr>
<tr>
<td></td>
<td>p 0.503</td>
<td>0.423</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*Duration of Employment</td>
<td>r 0.181</td>
<td>0.834</td>
<td>0.833</td>
<td>-0.750</td>
<td>0.830</td>
</tr>
<tr>
<td></td>
<td>p 0.463</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>**Smoking Index</td>
<td>r 0.193</td>
<td>0.351</td>
<td>0.458</td>
<td>0.399</td>
<td>0.618</td>
</tr>
<tr>
<td></td>
<td>p 0.403</td>
<td>0.119</td>
<td>0.037</td>
<td>0.073</td>
<td>0.003</td>
</tr>
<tr>
<td>Superoxide</td>
<td>r -0.762</td>
<td>-0.661</td>
<td>-0.671</td>
<td>--------</td>
<td>-0.615</td>
</tr>
<tr>
<td>dismutase (u/ml)</td>
<td>p &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>--------</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>r 0.755</td>
<td>0.724</td>
<td>0.716</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>(nmol/ml)</td>
<td>p &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>--------</td>
<td>------</td>
</tr>
</tbody>
</table>

* Duration of employment in years.
** Smoking index: (no. of cig./Day × duration of smoking per year)

Multiple linear regressions were done to assess the ability of smoking index and age so as to predict malondialdehyde level. The model is significant, explaining 54.2 % of the variability in malondialdehyde but duration of exposure is the only significant predictor in the model (β = 0.138, P < 0.05). It was done to assess the ability of smoking index, age and duration of exposure to predict urinary arsenic. The model is significant, explaining 37.8 % of the variability in urinary arsenic, but no significant predictors for urinary arsenic, Table 6.
Table 6. Multivariate regression analysis to test predictor for urinary arsenic and MDA levels among exposed workers

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>R square</th>
<th>Regression coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde</td>
<td><strong>Smoking index</strong></td>
<td>0.542</td>
<td>0.003</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>* Duration of employment</td>
<td>0.138</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>Urinary arsenic</td>
<td><strong>Smoking index</strong></td>
<td>0.738</td>
<td>0.004</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>Age in years</td>
<td>-1.086</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Duration of employment</td>
<td>2.363</td>
<td>0.083</td>
<td></td>
</tr>
</tbody>
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* Duration of employment in years.
** Smoking index: (no. of cig./Day × duration of smoking per year)

4. DISCUSSION

This study showed increased oxidative stress among workers exposed to arsenic in copper smelter. In the present study, we found that exposure to copper fumes during the smelting process causes some health hazards mainly respiratory and neurological hazards, as shown in Table 2. Our results agreed to a study by Ekosse et al. [23]; who investigated some of the respiratory tract related symptoms and diseases as chest pains and frequent coughing among residents within Selebi Phikwe, Botswana. There were ongoing nickel-copper (Ni-Cu) mining and smelting activities. They found that 33% of the study people complained of persistent chest pains; 49% complained of persistent, frequent coughing, frequent headaches and back pain. In accordance to the previous results Ekosse, [24], revealed that health hazards increased with closeness to the mine and the concentrator/smelter plant.

These health affections could be attributed to the emissions of particulate matter and sulfur oxides during copper smelting. Copper and iron oxides are the chief constituents of particulate matter, but other oxides such as arsenic, antimony, cadmium, lead, mercury and zinc may be also present along with metallic sulfates and sulfuric acid mist.

So, in our study, we measured air levels of copper and arsenic dust encountered in the process of smelting. Measurements of copper and arsenic dusts were below maximum allowable limits according to Egyptian law [21], (Table 1) and international regulations as the American Conference of Governmental Industrial Hygienists, [25]; Agency for Toxic Substances and Disease Registry, [26]. Our study results were consistent with Sińczuk-Walczak et al., [27], who studied the effect of arsenic on the nervous system in the copper smelting factory. They found that the prevalence of nervous system complaints as headache, muscular fatigue and numbness were higher among the exposed workers. They added that exposure to As concentrations within the threshold limit values (TLV) can induce subclinical effects on the nervous system, especially subclinical neuropathy. Using personal protective equipment regularly can decrease the direct contact with toxic chemicals and its effect on the exposed individual. In our work, using personal protective equipment (PPE) among exposed workers revealed that the minority of workers (35%) were using PPE. In our study the MDA concentration in the blood and the activity of SOD were determined in an attempt to establish whether the effect of a long-term exposure of workers to arsenic in secondary copper smelter may initiate lipid peroxidation or not. In this study, the mean values of serum copper, serum arsenic, urinary arsenic and Malondialdehyde (MDA) blood levels were found to be statistically significant higher, while the mean levels of superoxide dismutase enzyme activity were statistically significant lower among exposed workers compared to the control Table 3. These findings were in agreement with Escobar et al. [28],
who studied the issue of chronic arsenic exposure and oxidative damage in lymphocytes among smelter workers and control subjects. They found that the level of urinary arsenic and serum MDA were significant higher among smelters while the level of SOD is significantly lower. Recently, similar findings were reported by Malekirad et al. [29], who determined oxidative stress status as well as ferrous (Fe) and Copper (Cu) levels in blood in iron–steel workers. They found that the workers showed higher blood levels of lipid peroxidation and Cu. Lower total antioxidant capacities were found among workers than the control group. According to Nandi et al [30], the antioxidant response to arsenic seems to be time dependent since superoxide dismutase (SOD) and catalase (CAT) activities were shown to increase initially but later turned down after prolonged exposure. Imamoglu et al. [31], study the oxidative stress among welders. They revealed that plasma Cu level and SOD enzyme activities were increased significantly in welders compared to the control workers. They found that the difference in the range of MDA levels in the welder subjects was much higher than in controls. However, this difference cannot be regarded as statistically significant (p>0.05). Copper is capable of catalyzing the formation of reactive hydroxyl radicals through the decomposition of hydrogen peroxide via the Fenton reaction and depletion of glutathione [32]. Arsenic materials produced as a by-product during the smelting process.

As oxidative stress is related to increase in oxidation of metabolites and the release of ROS, so the exposure to arsenic and copper might be related to changes in antioxidative enzyme activity as SOD. In support of the above mentioned idea, we found a decrease in SOD enzyme activity among exposed workers than controls.

It was intensively investigated that many of the metal ions that are present in tobacco smoke are known to actively participate in the production of oxidative stress. Cigarette smokes contain 50 potent carcinogens, that include polycyclic aromatic hydrocarbons (PAHs), nicotine and other organic chemicals. They interfere with the balance between oxidative stress and antioxidant enzyme activities that cause release of ROS and free radicals during the oxidative metabolism in the body [33-35]. Although there was no statistically significant difference between exposed workers and control group as regards the frequency of smoking and the smoking status. We found that among smokers of both groups there were statistically significant differences among exposed workers compared to the control group as regards the mean values of serum copper, serum arsenic, urinary arsenic, superoxide dismutase enzyme activity and malondialdehyde blood levels, Table 4. As most of our workers were cigarette smokers. There was a synergistic effect between smoking and exposure to both dust and fumes during the copper smelting process. From what is mentioned above, In the current study oxidative enzyme is altered among exposed smoker groups in comparison to expose non-smoker and reverse in its concentration in the unexposed group. Our results were in accordance to Milnerowicz et al. [36], who found that there were high levels of cadmium, lead and arsenic concentrations observed in the blood and urine of copper foundry workers who smoked over 20 cigarettes per day. In the current study, we studied the relation between the intensity of smoking among the exposed group and the level of Cu, As, MDA, SOD in blood and As in urine; there were statistically significant positive correlations between the smoking index and each of urinary arsenic and serum malondialdehyde blood level, Table 5. Our results were in agreement with Bizon’ et al. [37], who found that there was a positive correlation (p = 0.009) between the number of cigarettes per day (the intensity of smoking) and the concentration of a marker of lipid peroxidation (MDA) due to occupational exposure to heavy metals in smelters. The level of malondialdehyde was approximately twofold higher in the plasma of the smelters compared to the control group. Also, there was a positive correlation between intensity of smoking and the concentration of urinary arsenic (r= 0.43; p< 0.005) and Cu/Zn SOD activities (r= 0.40; p= 0.005). There were statistically significant positive correlations between the age and each
of malondialdehyde levels in blood and urinary arsenic, however, there was a statistically significant inverse relationship between serum superoxide dismutase and age, Table 5. Our results were consistent with Bizon et al. [37], who observed that the elevation of MDA concentration positively correlated with the age of the smelter and arsenic ($p=0.001$). Also, they found that the elevation of urinary arsenic concentration positively correlated with the age of the smelter ($r=0.27$; $p=0.037$). Nonetheless, there was no significant correlation between SOD and age of workers. In this study, there were statistically significant positive correlations between duration of exposure and each of serum arsenic, urinary arsenic and malondialdehyde blood level and statistically significant inverse relation with SOD enzyme activity, Table 5. Our results were in resemblance with the results of, (Escobar et al [27], Nandi et al [30]) Metals like iron, copper, chromium, cobalt and vanadium undergo redox cycling reactions. On the other hand, the redox inactive metals, such as cadmium and arsenic. They showed their toxic effects via binding to sulphydryl groups of proteins and depletion of glutathione, resulting in the production of reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects [38-39].

Imamoglua et al. [31]; Chia et al. [35]; Bizon et al. [37]; reported that smoking habit was a significant contributor to the lipid peroxidation and disturbance in the oxidant–antioxidant balance. The oxidative damage seems to be affected by another factor as duration of exposure in heavy metal recovery workers. These results agreed with our findings in this study.

5. CONCLUSION AND RECOMMENDATIONS

In the present study, we concluded that oxidative stress biomarker as malondialdehyde (MDA) was significant higher among copper smelter workers. It was positively related to copper, arsenic levels and duration of exposure. In support of this idea, we found that SOD enzyme activity levels were lower in the highly exposed workers than in controls. This study suggested that the effects induced by oxidative stress may promote the development of health hazards with continued occupational exposure to copper fumes. There is a role of oxidative stress in triggering the processes that eventually lead to the clinical effects of arsenic on the respiratory and nervous system, that we found among the exposed workers. We recommend using malondialdehyde level and superoxide dismutase enzyme activity as indicators of oxidative stress among exposed workers. As oxidative stress induces DNA damage as well as lipid peroxidation products as MDA. One of the principal effects of oxidative damage to DNA is the DNA base modification. Moreover Arsenic can induce DNA strand breaks directly by reactive oxygen species on the DNA bases, or indirectly during the course of the base excision repair mechanism. Further studies are recommended on larger numbers of copper exposed workers to support our results. Cytogenetic monitoring by using a chromosomal aberration assay, micronuclei assay and sister chromatid assay in order to detect the genotoxic effects will be the best.

CONSENT

All authors declare that a written consent to share in the study and an approval to give blood samples from each participant were obtained after explaining to them the aim and the importance of the study.
ETHICAL APPROVAL

The study was first approved by the ethical committee of Occupational and Environmental Medicine Department, Cairo University, Egypt. Permission was obtained from the authorities of the secondary copper smelter factory in Helwan district in Cairo, Egypt, where the study was carried out. During the study, the ethical guidelines of good clinical practices (GCPs) have been explained. Strict confidentiality was observed throughout sample collection, coding, testing, and recording of the results.

CONFLICT OF INTEREST

There is no conflict of interest in this research.

REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
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