JCHR (2024) 14(3), 473-488

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Occupational Exposure to Heavy Metal Dust and Its Hazardous Effects on Non-ferrous Foundry Workers' Health

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(Received: 16 May 2024 Accepted: 31 July 2024)

KEYWORDS

Foundries;
Metals dust;
8-OhdG;
Blood metals; Oxidative stress

ABSTRACT: Exposure to metal dust is a significant occupational hazard for foundry workers. This study aimed to investigate exposure to potentially toxic metals and oxidative stress indices and assess the health risk of occupational exposure to metal dust among foundry workers. Environmental and biological exposures to a cocktail of metals were examined by measuring the concentration of Aluminum (Al), Zinc (Zn), Copper (Cu), Cadmium (Cd), Nickel (Ni), and Chromium (Cr) in the air of the workplace, as well as in the blood of the exposed workers. Malondialdehyde (MDA), reduced blood glutathione (GSH) and urinary 8- hydroxydeoxy guanosine (8-OH-dG) were measured as biomarkers of oxidative stress. All air measurements were below the maximum allowable limits (MAL) except for Al and Ni according to American Conference of Industrial Hygienists (ACGIH) and National Institute of Occupational Safety and Health (NIOSH). Here is significantly elevated Blood Al, Zn, Cu, and Pb levels in exposed workers. Moreover, MDA and 8-OHdG levels significantly increased ($P<0.0001$). In contrast, the mean level of GSH was reduced considerably in exposed workers compared to the control group ($P<0.0001$). The MDA acts as a marker with the highest Area Under the Curve (AUC), enabling effective differentiation between the exposed and control subjects (AUC = 0.968; Sensitivity = 90%, Specificity =100%; $P <0.0001$). Workers occupationally exposed to these metals for prolonged periods possessed higher metal levels in their bodies, which is associated with increased oxidative stress, which consequently causes DNA damage.

INTRODUCTION

Foundry factories are industrial establishments that melt and cast metals to produce a variety of shapes. Nevertheless, this procedure can produce perilous heavy metal particulate matter, which presents potential dangers to the well-being and security of personnel in the foundry sector[1].

Non-ferrous foundries are specialized facilities that focus on the casting and shaping metals that do not include iron. In contrast to ferrous metals, non-ferrous metals lack iron as their fundamental constituent[2]. These foundries specialize in working with a wide range of metals, including Aluminum, copper, Lead, zinc, nickel, brass, bronze, and other alloys. Non-ferrous foundries are essential in manufacturing industries as they provide

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DOI: 10.60829/jchr.2024.19934

diverse products. Their casting methods encompass a variety of processes, such as sand casting, investment casting, die casting and centrifugal casting, to fabricate complicated and long-lasting metal components. These foundries frequently cater to alloys, automotive, aircraft, construction, electrical, marine, and telecommunications industries. Non-ferrous metals have advantageous characteristics such as low weight, corrosion resistance, capacity to conduct electricity, and flexibility, rendering them appropriate for various uses[3].

Workers in foundry factories can be exposed to heavy metal dust such as Aluminum, Lead, cadmium, mercury, arsenic, and chromium by breathing it in, swallowing it, or coming into contact with contaminated surfaces through their skin[4]. The primary exposure method is inhalation since workers may breathe in airborne dust particles while engaging in tasks like handling raw materials, pouring molten metals, and cleaning or repairing equipment. Upon entering the body, these metallic elements can gradually amass and exert immediate and long-lasting impacts on health[5].

The immediate consequences of exposure to heavy metal dust can appear as discomfort, coughing, and difficulty breathing. Prolonged exposure, conversely, might result in significant health complications such as pulmonary impairment, renal dysfunction, neurological ailments, reproductive disorders, and potentially even malignancy[6]. Occupational exposure in non-ferrous foundries can arise from various sources, which may differ depending on the materials employed and work procedures, Figure 1). Some typical sources of such exposure include metal fumes, Dust and Particulate Matter, Chemicals and solvents, Noise (grinding, hammering, or metalworking) and Heat and Burns[7]. Heavy metal exposure in non-ferrous foundries may lead to adverse health effects for employees, such as Respiratory difficulties [8], Neurological disorders[7], Renal damage [9], Reproductive health [10] and Liver damage [11].

Oxidative stress occurs when there is an imbalance between the production of free radicals and the ability of cells to eliminate them. Excessive amounts of hydroxyl radical and peroxynitrite can lead to lipid peroxidation, damaging cell membranes and lipoproteins [12]. Heavy metals can produce reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) through chemical interactions in the body [13, 14]. Reactive oxygen species (ROS) can overwhelm the body's antioxidant defences by reducing the amounts of antioxidants such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and others [15]. Antioxidant defences play a critical role in counteracting reactive oxygen species (ROS) and preserving the equilibrium of redox reactions. ROS, or reactive oxygen species, assault cellular membranes, resulting in lipid peroxidation. This process entails the breakdown of lipids found in cellular membranes, producing lipid hydroperoxides, aldehydes, and other detrimental consequences[16].

Multiple studies have demonstrated that exposure to these metals leads to an imbalance between pro-oxidants and antioxidants and can mediate oxidative stress [17]. This is due to increased markers of lipid peroxidation levels such as Malondialdehyde (MDA), oxidative DNA damage (8-OHdG), DNA fragmentation, chromosomal damage, and the decrease in the activity of GSH enzymes, all of which could affect the degree of oxidative stress [18, 19]. This paper provides a study carried out in a non-ferrous foundry plant concerned with manufacturing aluminum, Lead, zinc, and copper products. The levels of metals in the air, whole blood, and urine were determined. Additionally, the oxidative stress parameters and risk assessment were investigated.

Aim of the work

The objectives of the present study were to evaluate the health hazards after exposure to heavy metal dust among non-ferrous foundry workers in Egypt including evaluations oxidative stress parameters, hematological profile, liver and kidney biomarkers. This was achieved by measuring the level of oxidative stress by malondialdehyde activity and the level of Reduced blood glutathione (GSH). the assay of 8-hydroxy-deoxyguanosine marker was used as a precursor of genome instability and DNA damage.

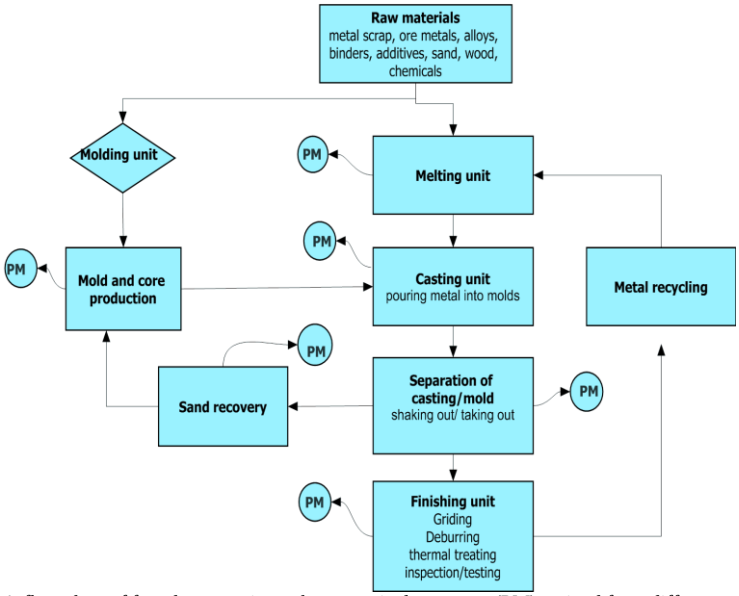

Figure 1. flow chart of foundry operations, shows particulate matter (PM) emitted from different units.

MATERIALS AND METHODS

Population and area of study

The data presented herein were gathered at a non-ferrous foundry plant in Helwan, Cairo, Egypt. The foundry plant produces various alloys of Al, Ni, Cu, Zn and other precious metal products**.** The study population included 90 subjects, including 60 males aged (25-58 years) who were working in different factory sectors for at least three years and occupationally exposed to these metals. The occupational history comprises current and previous jobs, nature of exposures, Duration of employment, number of hours exposed, and utilization of personal protective equipment such as masks or gloves. Occupational history stressed the workplace's surrounding environment, including ventilation, illustration, habits, clothing, crowdedness, and water supply. The study excluded individuals with a prior history of substance addiction, including drugs, smoking, and alcohol, as well as those with genetic disorders or severe medical conditions such as cardiovascular, renal, and hepatic disorders, diabetes mellitus, and cancer. The control group (G1) included 30 males aged (25-60 years) living far from the foundry plant and matched for age, smoking habit, and socioeconomic status. The control group had normal liver and kidney function tests and was never occupationally exposed to metal dust in any smelter processes in the past. Additionally, the exposed group (G2) was subdivided according to exposed Duration into two subgroups: (G2a) with a duration time of less than 15 years while (G2b) with a duration time of more than 15 years.

Demographic characteristics of the study groups

The demographic characteristics of the two studied groups (controls and exposed workers) were studied. The average age of the control and exposed workers were (36.2 ± 9.3), (37.7 ± 10.4) respectively. The mean average working time (years) was (17.4 ± 1.6) and the mean average of daily metal dust exposed time (hours) was 7.5 ± 1.4 among the exposed workers. The study excluded smoker individuals. None of the workers were observed using any form of personal protective equipment. They possessed poor socioeconomic status and exhibited little educational achievement.

Samples preparation and analysis

Air sampling

Metals were detected in the air of a particular division of the preparing plant. The assembling plant was made up of 2 departments: administrative and production. The production department contained various units, including

melting, moulding, casting, separation of (casting/mould), and finishing units. However, these units were not separated, and workers did not work in any specific unit but could work in different units at any time. According to the standard method provided by the National Institute for Occupational Safety and Health NIOSH Method 7300, air samples were gathered from the various production units using a pump connected with a filter cassette (Millipore cellulose ester filters) located in the breathing zone of workers during the whole working shift. An air sampling pump was operated for 8 hours, and twelve samples were collected from each selected site. The loaded filter was extracted by digestion with a mixture of 5 mL HNO_3, 65 % (Merck, Germany) and 2 mL HCl 36% (Merck, Germany) in a Pyrex beaker and let still for at least 2 hours at 95°C on a hot blade[20]. The extracted solutions were filtered using Whatman no. 40 filter paper and then made to 50 mL with deionized water; metals were detected and quantified by ICP-OES (Agilent 720).

Collection of blood and urine samples

Venous blood sample (5.0 mL) was collected from each worker using a dry plastic disposable syringe under complete sterilization conditions to measure exposed metals (Al, Cu, Cd, Cr, Ni and Zn) and oxidative stress biomarkers such as GSH and MDA. Each worker provided a urine sample in a clean polypropylene container to measure the 8-OHdG level.

Sample digestion

Blood samples (1.0 mL) were directly placed into an individual Teflon microwave digestion crucible. Subsequently, a total of 3 mL of a recently made solution containing concentrated HNO_3 and H_2O_2 in a ratio of 2:1 (V/V) was introduced into each Teflon crucible. The crucibles were covered and kept at room temperature (~25 °C) for three minutes (pre-digestion time), as illustrated in Figure 2. Subsequently, Teflon crucibles were introduced into a microwave oven and subjected to a one-stage digestion program at 600 W [21]. After completing the digestion process, all samples were taken out of the microwave and let to cool down to the ambient temperature (~0.5 mL). They were transferred quantitatively into 10 mL volumetric flasks and solutions made up to volume with deionized water. Samples were then transferred into a polyethene storage tube for further analysis [22, 23].

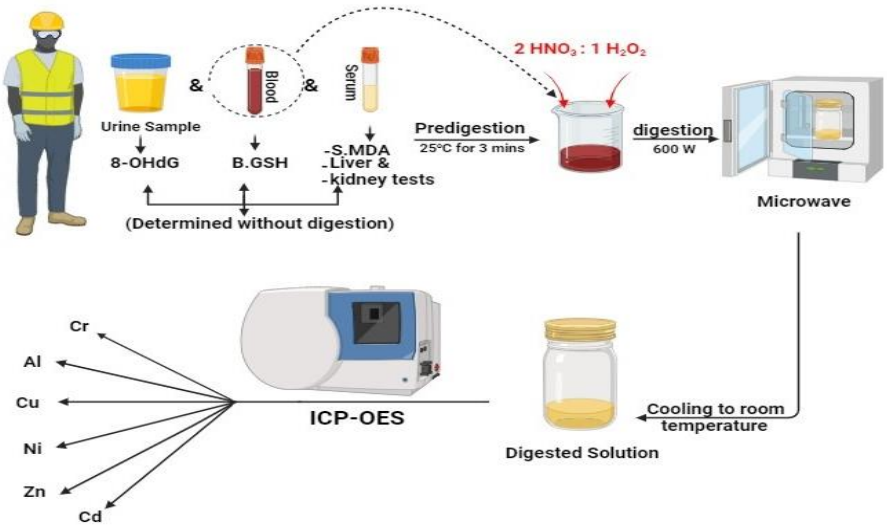

Figure 2. Schematic diagram for determination of MDA, GSH, 8-OHdG, Hematological, Liver, Kidney parameters and different metals in air and blood samples.

Determination of malondialdehyde (MDA) in serum

Malondialdehyde was measured colorimetric ally using Diagnostic and Research Reagents of Biodiagnostic,

Egypt. The reagents were supplied in the form of chromogen, enzyme-buffer and standard kit using the modified method by Ohkawa et al. [24].The Lipid Peroxidation (MDA) Assay Kit offers a straightforward, reliable, and standardized method for measuring lipid peroxidation in various biological samples such as blood, plasma, cell or tissue lysates, urine, or other body fluids. The assay employs the interaction between MDA and thiobarbituric acid (TBA), resulting in the formation of a pink-colored product. The magnitude of the pink hue, assessed at a wavelength of 532 nm, is directly correlated with the concentration of MDA present in the sample. The MDA-TBA adduct is subsequently quantified using calorimetry at a wavelength of 532 nm [24, 25]. The MDA concentration of unknown samples can be measured against standard sample using this calculation using semi-automated biochemistry analyzer BioSystems BTS-350; Barcelona, Spain.

MDA level in serum (nmol ml^{-1}) = Abs. of sample / Abs. of standard) × Standard conc.

Determination of reduced Glutathione (GSH) in the whole blood

The total glutathione content was determined using the glutathione reduced colorimetric method by using commercial kit from Biodiagnostic, Egypt. The colorimetric method was based on reducing 5, 5 dithiols, and 2-nitro benzoic acid (DTNB) with GSH to produce 2-nitro-5-thiobenzoic acid with yellow colour. The absorbance of the produced colour was assayed at 405 nm. Two test tubes were coded as sample and blank respectively. After adding specific amount of buffer and DTNB, all tubes were mixed well and the absorbance of unknown sample against the blank was determined after 5-10 min [26]. GSH was measured using semi-automated biochemistry analyzer BioSystems BTS-310; Barcelona, Spain.

GSH concentration in blood (mg dl^{-1}) = absorbance of sample × 66.66

Determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration in urine

This assay utilizes the competitive inhibition enzyme immunoassay approach. The microtiter plate included in this kit has been pre-coated with an antigen. Standards or samples are introduced into the suitable wells of a microtiter plate together with an antibody that specifically targets 8-OHdG and an enzyme called Horseradish Peroxidase (HRP) that is linked to a goat-anti-mouse antibody. The competitive inhibition response occurs between pre-coated 8-OHdG and 8-OHdG present in the samples. A substrate solution is introduced into the wells, and the colour intensity is inversely proportional to the concentration of 8-OHdG in the sample. The reaction is stopped by adding a solution of sulphuric acid, and the resulting colour change is quantitatively quantified using a spectrophotometer at a wavelength of 450 nm. The concentration of 8-OHdG in the samples is subsequently assessed by comparing the optical density (O.D.) of the samples to the standard curve. Calculate the mean of the repeated measurements for each standard and sample, then remove the mean optical density of the Blank. To create a standard curve, plot the average absorbance for each standard on the x-axis against the concentration on the y-axis. Then, draw a curve that best fits the points on the graph. To linearize the data, plot the logarithm of the 8-OHdG concentrations against the logarithm of the O.D. When samples are diluted, the concentration obtained from the standard curve needs to be multiplied by the dilution factor [27].

Measurement of hematological parameters among control and exposed groups

Blood sample was taken from participant and put into KEDTA tubes for complete blood count analysis. Complete blood count was performed Sysmex KX21 Hematology Analyzer, provided by Sysmex Corporation, Japan.

Instrumental analysis

Al, Cu, Cd, Cr, Ni and Zn metals were measured using an inductively coupled plasma emission spectrophotometer (720 ICP-OES, Agilent technologies). The sample was introduced by a concentric glass nebulizer attached to a cyclonic glass spray chamber, allowing for a single pass of the sample. A self-contained peristaltic pump with three separate channels and a

strong solid torch standard (2.4 mm axial ID injector) was used. The operating conditions of the ICP-OES were carefully chosen to maximize the sensitivity of the desired elements and achieve the highest precision and accuracy. ICP-OES operating parameter conditions are given as follows: RF Power (1.4 KW), nebulizers pressure (240 KPa), plasma gas flow rate (15 L min^{-1}), auxiliary gas flow rate (1.5 L min^{-1}), uptake delay of the sample (30 sec), instrument stabilization delay (15 sec), pump rate (15 rpm), rinse time (10 sec), replicates (3 times) and replicate read time (1 sec). Each element was measured at a specific Wavelength (nm). This emission intensity represents the concentration of each element within the samples [28]. A set of calibrated reference samples, ranging from 10 µg L^{-1} to 1000 µg L^{-1}, was created using the Agilent multielement stock standard solution (5 mg L^{-1}). A standard curve of a series of standard concentrations was created by plotting intensity against concentration (µg L^{-1}). Samples were analyzed simultaneously, and the concentration was calculated from the plotted curve (µg L^{-1}). A standard of known concentrations was analyzed for every ten samples to check for instrument drift.

Statistical analysis

The statistical analysis of the results was performed using the social sciences statistical software package (SPSS) version 19. Quantitative variables were used to estimate the mean values, standard deviations, and ranges. The student's t-test for data with normal distribution and nonparametric tests (Mann–Whitney test) were used to measure some quantitative variables. The Pearson correlation coefficient was utilized to calculate the correlations among individual variables. In order to express the Composite Exposure Index, a Principal Component Analysis was performed and the resulted F1 factor was assumed as representative of global exposure to aluminum, cadmium, chromium, copper, nickel and zinc in whole blood Statistically significant results were defined as having p-values < 0.05. MedCalc software was used to build the ROC curve for oxidative stress indices [29].

RESUTS AND DISCUSSION

Air monitoring inside the foundry plant

Twelve air samples were collected from the manufacturing department. The levels of Al, Cd, Cu, Zn, Cr and Ni in the workplace environment had a mean value ±SD (10.31 ± 3.73, 0.01± 0.006, 0.67±0.3, 1.14 ± 0.22, 0.05±0.02 and 0.07±0.04 mg m^{3-1}, respectively), with the total of suspended particles (TSP) 1.5 ±0.19 (mg m^{-3}), as shown in Tables 1 and 2.

Table 1. Statistical analysis of metals concentration (mg m^{3-1}) in airborne particles in the production unit of the foundry plant.

Metals (mg m^{3-1})	Mean± SD	Percent (%)	OSHA mg m^{3-1} PEL-TWA	ACGIH mg m^{3-1} TLV-TWA	NIOSH mg m^{3-1} REL-TWA	MAC mg m^{3-1}
Al	10.31±3.73	84.0 %	15	10	10	15
Cd	0.01±0.006	0.09 %	0.2	0.05	0.05	0.01
Cu	0.67±0.3	5.0 %	1	1	1	1
Zn	1.14±0.22	9.0 %	5	2	5	5
Cr	0.05±0.02	0.44 %	1	0.5	0.5	0.5
Ni	0.07±0.04	0.62 %	1	1.5	0.015	1

Table 2. Particle mass (mg m^{3-1}) in the different areas of the manufacturing department in the foundry plant (n= 12).

Manufacturing department	Mean	SD	Min	Max
TSP mg m^{3-1}	1.5	0.19	0.75	2.1

From previous data, the Al level was above the recommended exposure limit (REL) and threshold limit value (TLV) set by NIOSH (10 mg m^{3-1}) and ACGIH (10 mg m^{3-1}), and the Ni level was above the REL value set by NIOSH (0.015 mg m^{3-1}). However, the Cu, Cd, Cr, and Zn levels were below the REL value and TLV set by

NIOSH, ACGIH, and OSHA.

Biological monitoring

High levels of Al and Ni in workplace air were due to the generation of high amounts of metal dust and fumes combined with poor exhaust ventilation systems at the source of fumes. According to our previous measurements of the mentioned metals in workplace air, employees were exposed to them by inhalation during their work. Hence, these metals' levels should be measured in their blood, as shown in Table 3. Blood levels of the metals Al, Cu, Zn and Cd were observed in highly significantly increased concentration levels ((P<0.0001) in exposed employees compared to the control group. Ni and Cr blood levels were observed in highly non-significant increases (P>0.05) compared with the control group.

Likewise, copper levels in the blood were significantly higher in exposed workers than in the control group[30].

Table 3. Statistical analysis of blood Al, Ni, Cu, Zn, Cd and Cr (µg l^{-1}) in control and exposed groups.

Sample	Control (30)	Exposed (60)	P-value
B-Al (µg l^{-1})	7.21±3.18	58.3±18.43	0.0001
B-Ni (µg l^{-1})	59.87±11.78	62.45±17.44	0.693
B-Cu (µg l^{-1})	709.38±174.57	1308.4±126.4	0.0001
B-Zn (µg l^{-1})	4475.8±1201	6743.4±1449	0.0001
B-Cd (µg l^{-1})	0.92±0.31	1.37±0.72	0.0001
B-Cr (µg l^{-1})	3.57±0.67	3.62±0.93	0.807

Many agents can cause oxidative stress. The human body's cellular defense mechanisms can be divided into enzyme and non-enzyme. A balanced balance of oxidants and antioxidants leads to a healthy body. The word oxidative stress indicates that equilibrium and balance are disrupted [31].

In our study, serum MDA concentration and the activity of blood GSH were determined to establish the effect of employees' exposure to metal dust and whether it may initiate lipid peroxidation. In this study, the mean values of MDA and 8-OHdG were found to be higher with a high statistical significance (16.91 ± 3.87 nmol ml^{-1}), 37.48± 3.37 ng ml^{-1}, respectively with (P<0.0001). In contrast, the mean levels of GSH were lower among exposed workers compared to the control, with a high statistical significance of 18.87± 5.08 (mg/dl) (P<0.0001,) as reported in Table 4.

Table 4. MDA, GSH and 8-OHdG levels in the control and exposed groups.

Sample	Control (30)	Exposed (60)	P-value
MDA (nmol ml^{-1})	9.73± 1.62	16.91±3.87	0.0001
GSH (mg dl^{-1})	29.15± 4.18	18.77± 5.08	0.0001
8-OHdG (ng ml^{-1})	20.85± 3.28	37.48± 3.37	0.0001

There was a non-significant positive correlation between the composite exposure index and MDA with (p > 0.05), while there was a significant positive correlation with 8 OHdG with (P< 0.05). Simultaneously, there was a significant negative correlation observed between the composite exposure index and GSH with (p < 0.05), as reported in Table 5.

Table 5. Correlations between Composite exposure index with MDA (nmol ml^{-1}) & GSH (mg dl^{-1}) level and 8-OHdG (ng ml) levels among exposed workers.

Oxidative stress marker	Composite exposure index	
	r	P-value
MDA (nmol ml^{-1})	0.251	0.085
GSH (mg dl^{-1})	-0.404	0.004
8-OHdG (ng ml^{-1})	0.747	0.0001

Oxidative stress arises from a disparity between the generation of reactive oxygen species (ROS) and the protective mechanisms of the antioxidant defence system [16]. ROS can lead to an assault on several cellular components, including antioxidant defences, DNA, and membrane phospholipids; the latter is particularly vulnerable to oxidation, resulting in the generation of peroxyl radicals and, subsequently, malondialdehyde (MDA)[32]. According to our study, when there was an elevation of metals (Al, Cu, Cd, Cr, Ni and Zn) in blood workers, these metals can generate free radicals through different mechanisms, consequently increasing the rate of lipid peroxidation, which is measured as MDA levels, as intracellular levels of free radicals rise, there is a corresponding increase in MDA levels. In response to oxidative stress, the organism mounts a considerable increase in antioxidant activity to protect itself as a protection mechanism[33]. It was reported that there was a significant increase in MDA and a significant decrease in glutathione (GSH) in cement workers compared with control groups[34]. Another study revealed higher oxidative stress in zinc recovery plant workers than in secondary copper smelting plants[35]. Our findings agree with those reported by [36], who found elevated urinary 8-OHdG concentrations in foundry workers occupationally co-exposed to metals and polycyclic aromatic hydrocarbons (PAHs). Heavy metal exposure can cause an increase in oxidative DNA damage, as reported by[37]. Urinary 8-OHdG concentration was significantly correlated with the combined metals exposure index in working children compared with school children [38]. The present study examined the effects of multiple metal exposures by calculating the composite metal exposure index, as the recruited subjects were exposed to several metals. A strong positive connection was observed between 8-OHdG and the composite metal exposure index ($r = 0.747$, $p < 0.0001$), as previously reported by[29].

Mechanism of oxidative stress caused by heavy metals exposure

Multiple studies have demonstrated that oxidative stress in living cells arises from an equilibrium between creating free radicals and forming antioxidants, which are responsible for neutralizing the reactive substances or mending the consequent harm. Figure 3 depicts the impact of heavy metals on a cell and the equilibrium between the generation of reactive oxygen species (ROS) and the subsequent protection provided by antioxidants. Cellular antioxidants, such as glutathione, shield the cell from harmful free radicals such as superoxide anion $O_2^{\bullet-}$, hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2). When exposed to heavy metals, the amount of reactive oxygen species (ROS) in the body increases, while the level of antioxidants drops, Figure 3.

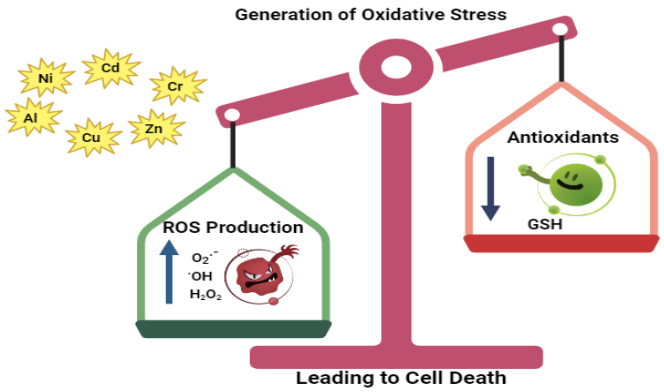

Figure 3. Mechanism of oxidative stress caused by heavy metals exposure

Statistical analysis revealed that the mean concentration of Hemoglobin (Hb) was decreased significantly in the exposed group (P<0.019) compared to the control group. In contrast, no statistically significant differences were found between the two groups as regards WBCs, RBCs, platelets count and hematocrit percentage, Table 6.

Table 6. The mean and standard deviation of the hematological parameters among the control and exposed group.

	Control (n=30)	Exposed (n=60)	T-test	P-value
	Mean± SD	Mean± SD		
Hb (g dl^{-1})	14.7±1.04	14.14±1.17	2.39	0.019
WBCs ×10^3. cmm^{-1}	7.55±1.9	7.79±2.18	1.59	0.624
RBCs ×10^6. cmm^{-1}	5.08±0.3	5.17±0.45	-0.91	0.362
HCT %	42.72±2.7	42.20±3.4	0.743	0.459
Platelets x10^3. cmm^{-1}	248.93±50.2	240.09±57.3	0.720	0.473

Statistical analysis revealed that the activity of AST, ALT, ALP and γGT significantly elevated in the exposed group at (P<0.004), (P<0.006), (P<0.006), (P<0.0001) respectively when compared with the control group. At the same time, levels of T.P and Albumin significantly decreased in the exposed group at(P<0.008) and (P<0.008), respectively, when compared with the control group. The level of urea and uric acid significantly increased in the exposed group at (P<0.025), (P<0.0001), but no significant difference for creatinine in the exposed group when compared with the control group at (P<0.644). In contrast, the activity of LDH significantly increased in the exposed group at (P<0.0001) when compared with the control group, Table 7.

Table 7. Liver and Kidney function tests in the control and exposed group.

	Control (n=30)	Exposed (n=60)	T-test	P-value
	Mean± SD	Mean± SD		
AST(U L^{-1})	27.21±6.61	33.55±9.23	-3.26	0 .002
ALT(U L^{-1})	24.6±4.67	30.76±10.96	-2.84	0.006
ALP(U L^{-1})	73.1±14.52	81.22±19.08	-1.99	0.049
T.P(g dl^{-1})	7.45±0.47	7.18±0.4	2.71	0.008
Alb.(g dl^{-1})	4.56±0.36	4.19±0.68	2.69	0.008
γGT(U L^{-1})	23.24±7.14	36.44±15.09	-4.39	0.0001
LDH (U L^{-1})	249.6±52	504.3±117	-10.97	0.0001
Urea(mg dl^{-1})	29.1±5.21	32.5±6.98	-2.3	0.025
Creatinine(mg dl^{-1})	0.79±0.15	0.81±0.2	-0.46	0.644
U.A (mg dl^{-1})	4.8±1.2	6.52±1.3	-5.9	0.0001

Synergistic effects of the exposure to a cocktail of metals (Al, Cu, Cr, Cd, Ni & Zn) on biochemical parameters level among exposed workers

Correlations between composite exposure with liver and kidney parameters showed a positive correlation with AST, ALT, ALP, Alb, γGT, LDH, Urea, Creatinine and Uric acid with P< (0.001, 0.044, 0.91, 0.125, 0.314, 0.136, 0.021, 0.425, 0.307), respectively. At the same time, there was a negative correlation with Total protein (P<0.248), as shown in Table 8.

Table 8. Correlations between Composite exposure index with Liver and kidney parameters

	Composite exposure index	
	R	**P-value**
AST(U L^{-1})	0.446	0.001
ALT(U L^{-1})	0.286	0.044
ALP(U L^{-1})	0.016	0.91
T.P(g dl^{-1})	-0.166	0.248
Alb.(g dl^{-1})	0.221	0.125
γGT(U L^{-1})	0.145	0.314
LDH (U L^{-1})	0.214	0.136
Urea(mg dl^{-1})	0.325	0.021
Creatinine(mg dl^{-1})	0.156	0.425
U.A (mg dl^{-1})	0.147	0.307

Duration of exposure

The exposed group (G2) was divided into two subgroups based on the Duration of exposure to metal dust to study the relative relation between the Duration of exposure to metal dust and metal level. G2a (including 34 male workers) had less than 15 years of exposure to metal dust, and G2b (including 26 male workers) had more than 15 years of exposure. G1 included 30 males. Statistical analysis revealed that the mean MDA level and median 8-OHdG show a high increase in exposed groups G2b more than G2a with a high significance of P <0.0001 compared with the control group G1. There was also a significant difference between the exposed groups G2a and G2b (P<0.05)**.** Statistical analysis revealed that the GSH level decreased in exposed groups G2b less than G2a compared with the control group G1 with a significance of P<0.01. At the same time, there was a significant difference between exposed groups G2a and G2b at P<0.05, Figure 4.

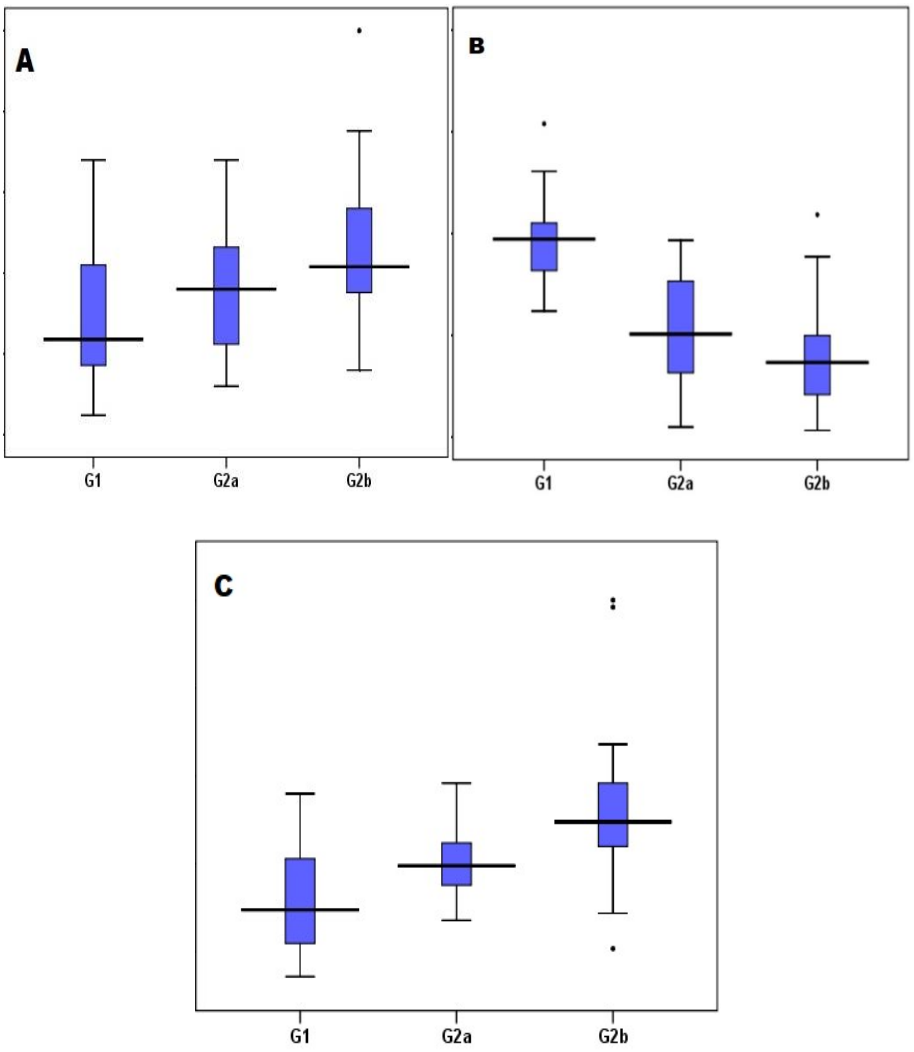

Figure 4. Box blot for Sera median concentrations of (A) MDA (nmol mL^{-1}), (B) GSH (mg dl^{-1}), and (C) 8-OHdG(ng ml^{-1}) in the control and different exposed groups.

Diagnostic validity of the single markers

ROC curves were developed to assess the diagnostic efficacy of specific oxidative indicators. The MDA serves as a marker with the highest Area Under the Curve (AUC), enabling effective differentiation between the exposed and control subjects (AUC = 0.968; Sensitivity = 90%, Specificity =100%; P <0.0001). At the same time, AUC for GSH was (0.936; Sensitivity= 75%, Specificity = 100%; P<0.0001), and AUC for 8-OHdG was (0.936; Sensitivity= 60.29%, Specificity = 96.43%; P<0.0001), respectively as shown in Table 9 and Figures 5 and 6. Therefore, MDA may be used as an oxidative stress marker to follow up with exposed workers.

Table 9. Diagnostic sensitivity and Specificity of MDA, 8-OHdG and GSH to distinguish between controls from exposed workers.

	Control vs. exposed		
	MDA	**GSH**	**8-OHdG**
Cutoff	>12.2	≤22.33	>29.9
AUC	0.968	0.936	0.816
SE	0.0156	0.024	0.040
P-value	<0.0001	<0.0001	<0.0001
Sensitivity %	90	75	60.29
Specificity %	100	100	96.43
PPV %	100	100	97.9
NPV %	82.4	65.1	46.6
Youden index	0.9	0.75	0.566

AUC: Area Under Curve; SE: Standard error; PPV: Positive Predictive Value; NPV: Negative Predictive Value.

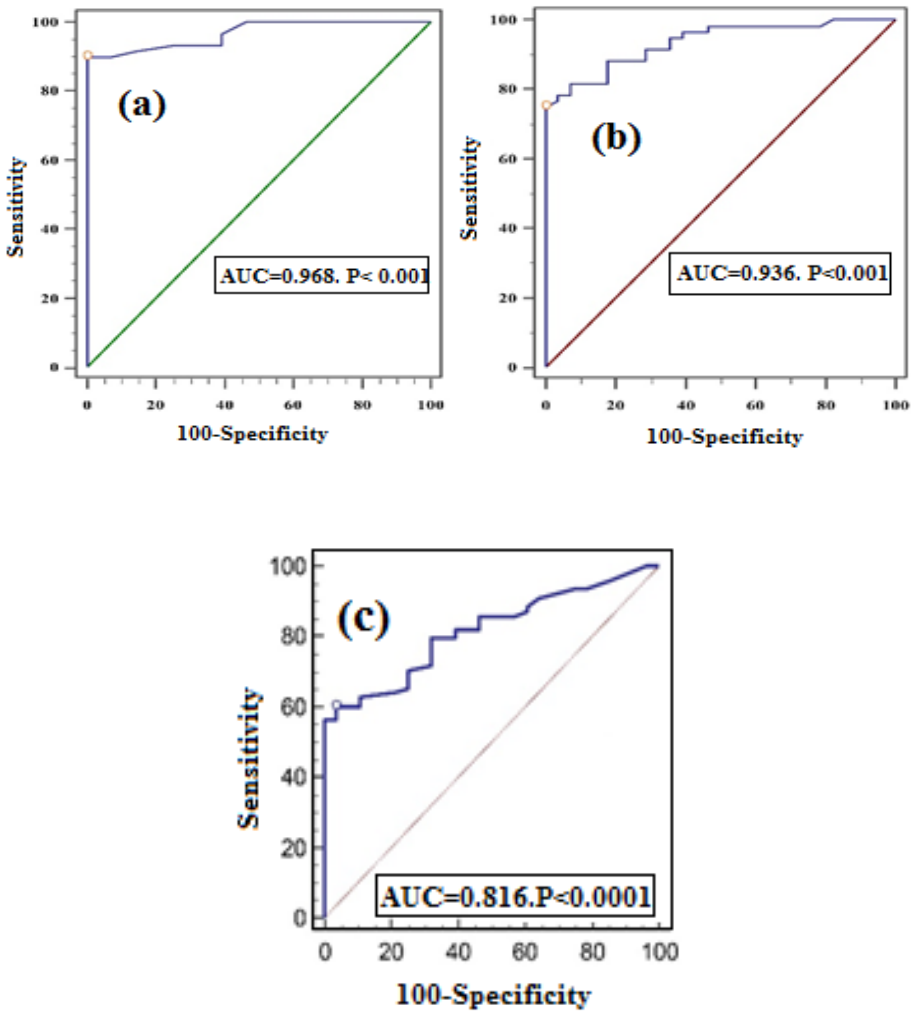

Figure 5. ROC curves for (a) MDA, (b) GSH and (c) 8-OHdG to discriminate exposed workers from controls. AUC: Area under curve.

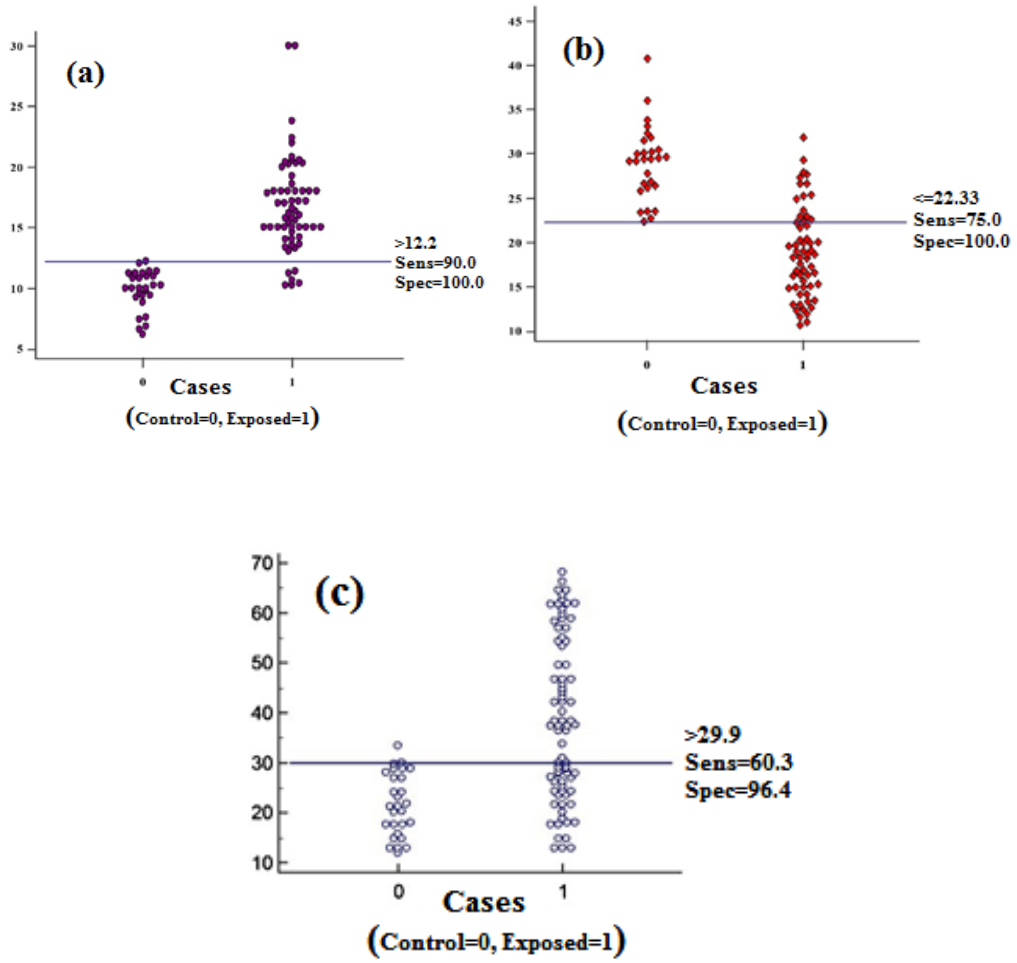

Figure 6. Interactive dot diagram for (a) MDA, (b) GSH and (c) 8-OHdG to discriminate exposed workers from controls. Sens: Sensitivity; Spec: Specificity.

CONCLUSIONS

Our research has shown that employees are exposed to metal dust close to normal limits set by some regulatory agencies, which causes metals to rise to a safe level in their blood. However, with time, these metals tend to accumulate and cause oxidation. Workers need to track this level, while risks can be managed through protective equipment and control measures for engineering and education policies. The results show that MDA is the most useful oxidative marker that might be used to monitor workers' health. The investigated workers are exposed to relatively high levels of Al and Ni and a considerable amount of Cu, Cd, Zn and Cr in their workplace. Workers occupationally exposed to these metals for prolonged periods possessed higher metal levels in their bodies, which is associated with increased oxidative stress, which consequently causes DNA damage and elevation of liver and kidney biomarkers with the decrease of hemoglobin levels compared to the non-exposed group. To minimize the hazards linked to exposure to heavy metal dust, foundry facilities should adopt safety measures, including installing effective ventilation systems, using personal protective equipment (PPE) such as respirators and gloves, and regularly monitoring air quality. Furthermore, workers must undergo enough training in safely handling heavy metals and receive comprehensive education regarding the potential health hazards they may encounter.

ACKNOWLEDGEMENTS

The authors thank the Committee of the National

Research Centre in Egypt (NRC) that approved this study and the participants who enrolled in the study.

ETHICAL CONSIDERATION

The Ethics Committee of the National Research Centre in Egypt (NRC) approved this study, with registration number 15225. Obtaining informed consent was ensured from all participants who participated in the study. Data confidentiality was guaranteed.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

Consent for publication

Not applicable.

Availability of data and material

All data in the manuscript were available with the corresponding author upon request

Funding:

Not applicable.

Author contributions

Author Contribution: Y.F. and A.E. drafted and wrote the manuscript. Y.F. and I.H. prepared tables and collected samples, H.A. prepared figures, N.L. and N.S. edited and corrected the manuscript. All authors reviewed the manuscript.

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