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Lead and cadmium induce chromosomal aberrations and DNA damage among foundry workers

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ABSTRACT

Heavy metals are considered a major occupational hazards in foundry industry, and exposure may result in cytotoxic, genotoxic and DNA damage effects. This study aims to assess blood metal levels namely (Pb, Cd, Cr, Mn, and Ni) and to elucidate their association with blood selenium levels. Moreover, to evaluate cytogenetic alterations and DNA damage along with hematological parameters in foundry workers. This case–control study included 26 male workers (exposure group) and 15 male controls. The biological exposure to cocktail of metals was determined via measuring the blood levels of lead (B-Pb), cadmium (B-Cd), chromium (B-Cr), manganese (B-Mn) and nickel (B-Ni), as well as selenium (B-Se) by inductively coupled plasma optical emission spectrometry (ICP-OES). Additionally, composite exposure index was calculated. Chromosome aberration (CA) and DNA fragmentation were exploited to detect genotoxicity. Hematological parameters were determined on an automated hematology analyzer. Blood metals analysis unveiled significantly elevated levels of B-Pb and B-Cd (P = 0.006; 0.012 respectively), while B-Se level was significantly lower in exposed workers as compared to controls (P = 0.017).The frequency of DNA fragmentation was found to be significantly higher in the exposed group (P = 0.018). Whereas, no statistically significant difference was found between the two groups in regards to CAs, however, the higher frequency of CAs among exposed workers than in controls (23.08%) was found. The level of hemoglobin markedly decreased in workers as compared to controls (P = 0.012). The multiple regression analysis showed that the composite exposure index highly affect the frequency of DNA fragmentation patterns, while B-Pb was the only variable affect the level of B-Se with coefficient of determination (R^2 = 0.524; 0.426, P = 0.0001; 0.0001 respectively) among the exposed workers. Taken together, we revealed that the co-exposure to lead and cadmium induced DNA fragmentation and increased chromosomal aberrations in foundry workers.

Key words: foundry workers, DNA fragmentation, CAs, ICP-OES**,** composite exposure index

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INTRODUCTION

Foundry work involves two main procedures: sand molding and metal casting. Sand molding includes core sand and binder, core making and the casting process which comprises essentially of pouring molten metal into a mold containing an attachment into the geometry desired for the final part of the procedure. After casting cooling, shakeout and cleaning, the metals and sand are recouped[1], [2]

During these process, foundry workers may be exposed to various hazards such as particulate matter, silica, polycyclic aromatic hydrocarbons (PAH), high temperatures and machinery[3].

Particulate matter in foundries has variable composition; however, lead (Pb) and cadmium (Cd) are elements of concern for nonferrous alloys because they are minor components of most processed brasses. Even in the modern foundry plants that adopt the most advanced tools and procedures for reducing exposure to toxic metals, workers are occupationally exposed to higher levels of airborne toxic agents (metals) than those found outdoors [4]–[7].

Previous studies on nonferrous foundries have documented overexposure to lead and cadmium from the melting and casting of molten metal alloys. [7], [8].

Lead is recognized to be highly toxic and can cause acute or chronic health impacts. Though it can affect various organs in the body, it mainly leads to toxicity of the cardiovascular, hematopoietic, nervous, and other systems[9]–[11]. In addition, recent study by Ilychova and Zaridze[12]have been demonstrated an association between lead exposure and both pancreatic and kidney cancer mortality. Lead is classified as a class 2A carcinogen (i.e. probably carcinogenic to humans)[13].

It has been well known that Pb can interacts and competes with some essential elements[14]. One of them is selenium (Se). Se is anon-metal element that has been connected to numerous medical advantages in humans and experimental animals, for example, decreasing the incidence of cancer, protecting against cardiovascular diseases, and treating certain muscle disorders[15], [16].Moreover, selenium (Se), assumes as an antioxidant [17], since Se is a cofactor of glutathione peroxidase and decreases the levels of lipid peroxidation. It plays important role in protecting DNA, RNA and proteins from oxidative damage. Additionally, Se can binds to lead and forms inactive selenium–lead complexes [18] and, consequently, reduces the amount of free lead ions in the body [19].

Cadmium is known to have numerous undesirable effects on human health, the main organs affecting by cadmium toxicity including kidneys [20] and liver [21], additionally may effects other organs/systems as reproductive [22] and skeletal systems [23].Cadmium and its compounds are recognized as human carcinogens depended on sufficient experimental and epidemiological evidence. Cadmium can cause genotoxic effect, induce DNA damage and lead to apoptosis in a variety of eukaryotic cell types, including human cells. Cadmium is classified as Group 1 (i.e. carcinogenic to humans) according to the International Agency for Research on Cancer[13].

The aim of the present study was to assess blood metal levels (Pb, Cd, Cr, Mn, and Ni) and to explore their association with blood selenium level. In addition, to evaluate cytogenetic alterations by CAs and DNA damage by DNA fragmentation assay along with hematological parameters in foundry workers occupationally exposed to lead and cadmium.

EXPERIMENTAL SECTION

This study included 26workers occupationally exposed to lead and cadmium in lead casting department, and 15 healthy individuals age matched who had never worked in foundry-related jobs served as control group.

All participants in this study were interviewed and completed questionnaire including demographic data, smoking statues, duration of employment, exposure hours, vacations, and usage of personal protective equipment as masks or gloves. A written consent for all participating was taken according to the declaration of Helsinkki. This study was approved by the ethical committee of the National Research Center (NRC).

The exclusion criteria in this study was as follow: hypertension, diabetes, having any chronic illness, receiving any medical treatment during the last six months especially the antioxidant drug and presence of any genetic disorders.

Blood samples were collected from the included participants between 12 and 2 p.m. before the lunch break on the Thursday as the finishing of the weekly work shift. Venous blood (10 ml) was collected using a dry plastic disposable syringe under complete aseptic condition and divided into three parts: the first and second parts (3 and 4 ml respectively) was collected into KEDTA tubes for hematological parameters, DNA isolation and metal analysis, and the third part (3 ml) was collected into heparinized tubes for cytogenetic study.

1-Metal analysis

1.1. Reagents and glassware for metal analysis:

Ultrapure HNO_3 (d = 1.39 g/cm^3 (20°C)) from Merk (Germany), 30% (m/m) H_2O_2 (d = 1.11 - 1.45 kg l−1), an Agilent multi-element stock standard solution (5 mg/l) were used. Calibration standard solutions were obtained from the stock solution by suitable dilutions. All solutions were stored at −5°C until needed for analysis.

1.2. Digestion of blood samples

The method used in the present study was based on the methods of [24], [25] with some modification in digestion time and microwave oven program. The procedures were as following: An aliquot of 0.5 ml of whole blood from each subject was directly placed into a Teflon microwave digestion vessel, and 3 ml of mixture contain HNO_3 − H_2O_2 (2:1, v/v) was added to each Teflon crucible. The later were covered and kept at room temperature (~25 $^{\circ}$C) for about 3 minutes as a pre-digestion time, then placed in a microwave oven. Crucibles were heated following a one-stage digestion program at 30% of total power (900 W) for 2-3 minutes till complete digestion. At the end of digestion, all samples were removed from the microwave and allowed to cool at room temperature, the resulting solution (about 0.5 ml) was diluted with deionized water (D.W) up to 10 ml and transferred to a polyethylene storage tube for further analysis. Reagent blanks were prepared by addition of deionized water in place of the samples and the same procedures were applied

1.3. Instrumentation

The measurements of B-Pb, B-Cd, B-Cr, B-Ni, B-Mn and B-Se were performed using a simultaneous inductively coupled plasma emission spectrometer (ICP-OES-720) (Agilent technologies).

Samples were introduced via glass concentric nebulizer fitted to glass cyclonic spray chamber (single pass). An independent three-channel peristaltic pump was used for pumping the sample. High solid torch Standard (axial 2.4mm id injector) was used. The ICP-OES operating conditions were well optimized and carefully selected for maximizing the sensitivity for the desired elements and to obtain the best precision as well as, the accuracy. Each element was measured at suitable wavelength that gives maximum sensitivity. The selected wavelength for selected element (Pb 220.353 nm, Cd 214.439 nm, Cr 267.716 nm, Ni 231.604 nm, Mn 257.610 nm and Se 196.026 nm) with plasma argon flow rate of 15 L/min, auxiliary gas flow rate of 1.5L/min, nebulizer pressure of 240 KPa, sample uptake delay 30 s, radio frequency power was 1.4 KW and Instrument stabilization delay was 30 s.

2. Hematological analysis

Red blood cells (RBCs), white blood cells (WBCs) count and hemoglobin levels were determined on KX-21 Sysmex automated hematology analyzer (Sysmes Corporation, Japan).

3. Cytogenetic method

Conventional structural CA analysis was applied according to the method described by Verma and Babu (1995), lymphocytes cultures were established within 7 h of sampling by placing 0.5 ml of PRMI medium and 20 % calf serum as well as, 1.5% Phytohaemagglutinin (FITO KRKA, Cairo, Egypt). Cultures were incubated at 37 °C for 48 h. After 46 h of incubation time 0.1 mg/ml of Colchicine solutions was added and left for 2 hours to stop mitosis at metaphase and prevent spindle formation. The cells were sedimented by centrifugation at 1000 rpm for 10 minutes. The supernatant fluid was removed leaving about 0.5 ml of it above the cell sediment and 5 ml of hypotonic solution (0.58% KCl) was added for swelling the cells and separating the chromatids. Carnoy's fixative (1:3 of glacial acetic and methanol) were added to culture**s.** At the final preparation step, the slides were stained by 5% Giemsa solution and analyzed microscopically. One hundred metaphases/person for CAs were analyzed and the results of structural or numerical anomalies were recorded.

4. DNA fragmentation analysis

Genomic DNA was isolated from whole blood of each participant using commercial kits according to the manufacturer's instructions (Thermo Fisher Scientific Inc., Waltham, USA). DNA fragmentation patterns were determined by agarose gel electrophoresis (2% agarose gel contain 0.5 µg/ml ethidium bromide)[26]. The electrophoresis was used to separate DNA fragments for 1.5 h at 100 V. The DNA ladder and the fragmentation patterns for controls and workers were visualized using UV trans-illuminator (Bio Rad, UK).

5. Statistical analysis

All statistical analyses were conducted using a statistical software package "SPSS 16.0 for Microsoft Windows, SPSS Inc., Chicago, Illinois, USA) and considered statistically significant at a two-sided $P< 0.05$. Numerical data were expressed as mean ± SD. Qualitative data were expressed as frequency (number=n) and proportion (%). Comparisons of quantitative data between two groups were done using the Student's t test for data with normal distribution and nonparametric tests (Mann–Whitney test) for data not assuming normal distribution as in case of blood metals. The chi-square (x^2) tests were used for qualitative variables. The correlation was evaluated by Pearson and spearman correlation coefficient. 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 lead, cadmium, chromium, nickel and manganese in whole blood[27]. Box blot was used to illustrate the difference in blood metals between studied groups. Box by itself represent the median and (25% to 75% percentiles) of the data.

Multiple linear regression analysis was used to estimate the influence of independent variables such as age, smoking status, duration of exposure and blood metals on the markers studied (dependent variables).

RESULTS

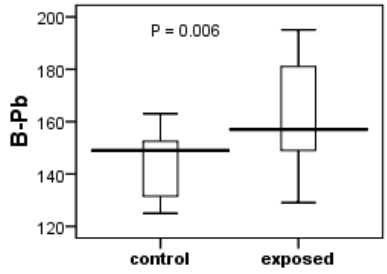
The demographic and other data of the control and exposed groups are presented in Table 1. The mean age of the participants in the control and exposed groups was (40.4 ± 6.65) and (44.92 ± 9.67 years, respectively). There was insignificant difference between the two studied groups as regards to age (*P* >0.05). The average years of employment in exposed group were23.84 ± 10.52years. No statistically significant difference was found between the exposed and control participants in terms of smoking habits (i.e. smokers and nonsmokers) (*P* = 0.730).

Table (1): demographic characteristic of controls and workers exposed to metals under investigation

Parameters	Control (n=15)	Exposed (n= 26)	*p* -value
Age (years)[a] Range	40.4 ± 6.65 33-53	44.92 ± 9.67 29-57	0.117[b]
Duration of employment (years)[a] Range	- -	23.84 ± 10.52 8 -36	-
Non-smoker [n (%)]	10 (66.67)	19 (73.08)	0.730[c]
Smoker [n (%)]	5 (33.33)	7 (26.92)	

[a] *Mean ±SD,* [b] *Students t-test,* [c] *Chi-square test.*

Fig. (1) Show the levels of B-Pb, B-Cd, B-Cr, B-Mn, B-Ni and B-Se of workers and controls. Results showed a significant increase of both B-Pb (Fig. 1A) and B-Cd levels (Fig. 1B) (P = 0.006 and P = 0.012 respectively), while B-Se level (Fig. 1F) was significant decreased in exposed group as compared to control one (P = 0.017). The rest of studied blood metals (Fig. 1C, 1D and1E) showed nonsignificant difference between the two groups (P >0.05).



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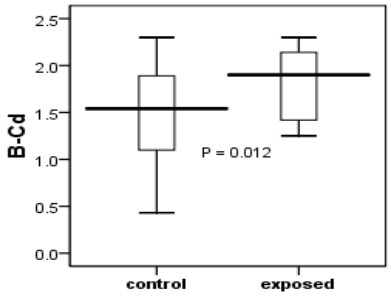

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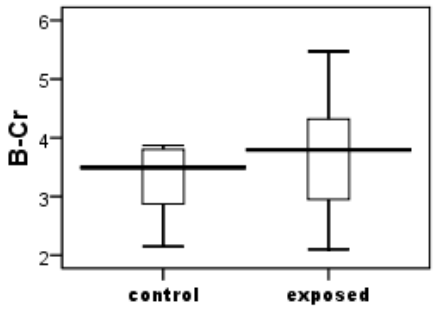

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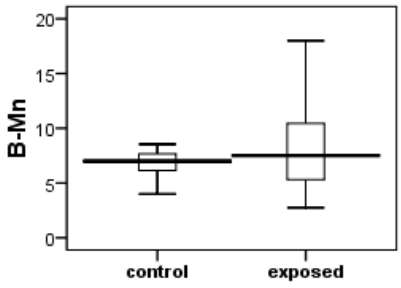

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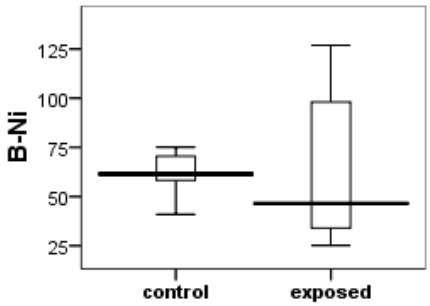

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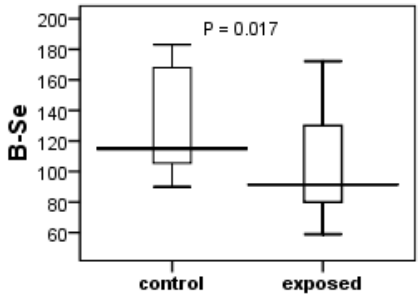

F

Figure 1: B-Pb, B-Cd, B-Cr, B-Mn, B-Ni and B-Se levels in control and exposed groups. Due to non-parametric distribution, results are presented as box plot, which represent the median and (25% to 75% percentiles) of the data

The prevalence of (CAs) were not statistically significant difference between the exposed workers and controls (*P*=0.070). However, the higher frequency of CAs among exposed workers than in controls (23.08%) was presented in this study, table 2.The different types of CAs manifested among the exposed groups are presented in table 3. There were three of workers out of six showed breakage in chromosome 1, two showed breakage in chromosome 3 and one showed breakage in chromosome 3 and 10.

Table 2: Frequency of Chromosomal Aberrations (CAs) and DNA fragments manifested among both exposed workers and controls

		Group				$\chi 2$	*p*–value
		Control		Exposed			
		N	%	N	%		
CAs	Normal	15	100	20	76.92	4.05	0.070
	Abnormal	0	0	6	23.08		
DNA fragment	positive	0	0	8	30.77	5.73	0.018
	negative	15	100	18	69.23		

Table 3: Characteristics of chromosomal Aberrations (CAs) among the studied groups

Characteristics of (CAs)*	Group			
	Control n=10		Exposed n=26	
	N	%	N	%
46 , XY Normal	10	100%	20	76.92%
46 , XY - breaks in Chr. ** (1)	0	0%	3	11.53%
46 , XY - breaks in Chr. (3)	0	0%	2	7.69%
46 , XY - breaks in Chr. (3,10)	0	0%	1	3.84%

CAs*: Chromosomal aberrations - Chr. **: Chromosome

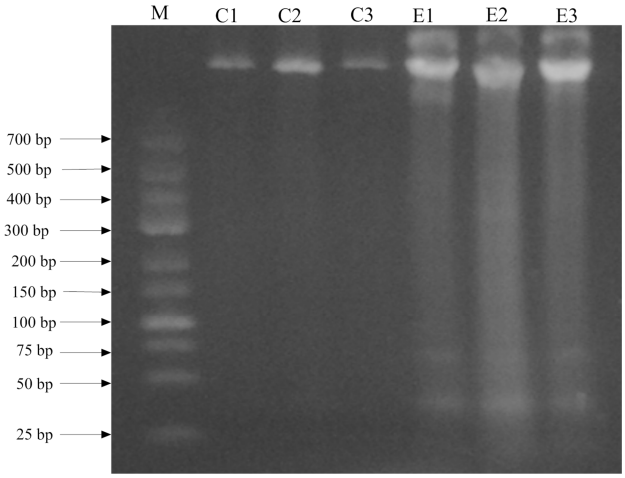

Figure 2: Shows the DNA fragmentation patterns using the agarose gel electrophoresis. The lane M shows the DNA marker with 25 base pair ladder, lane C1-C3 show the DNA bands in controls while lanes E1-E3 show DNA fragmentation patterns in the exposed subjects

The frequency distribution of DNA fragmentation, as indicator for the presence of DNA damage induced by exposure to multiple metals, among those workers in foundry plant and the control group are presented in table 2. Analysis of data revealed a significant increase of DNA fragmentation patterns in the exposed workers as compared to controls (P = 0.018). The genomic DNA damage was induced in workers exposed to lead and cadmium was presented in figure 2.

Table 4 shows that levels of RBCs and WBCs were not significantly difference in the workers and controls (P= 0.241; 0.791 respectively) but the level of hemoglobin markedly decreased in workers as compared to controls (P = 0.012).

Table 4: hematological parameters in controls and exposed workers

	Group			
	Control	Exposed	*t*-test	*p*-value
	N=15	N=26		
RBCs×10^6/µl	5.16±0.26	5.02±0.39	1.191	0.241
WBCs×10^6/µl	7.34±2.11	7.53±2.11	-0.267	0.791
Hbg/dl	14.53±0.58	13.73±1.09	2.62	0.012

Table 5 shows that, there were significant positive association between DNA fragmentation patterns with duration of employment, smoking habits, B-Pb, B-Cd, B-Cr and composite exposure index (P = 0.010; 0.005; 0.0001; 0.001; 0.011 and 0.0001 respectively), while significant negative association with B-Se (P = 0.027) was found among the exposed workers. Also there were significant negative correlation between B-Se with duration of employment, smoking habits, B-Pb, B-Cr and composite exposure index (P = 0.029; 0.021; 0.0001; 0.007 and 0.0001 respectively).

Table 5: the relations between DNA fragmentation and blood selenium with different variables among the exposed participants

Variable	DNA fragmentation	B-Se (µg/L)	Haemoglobin
	r[a]	r[b]	r [b]
Duration of employment (years)	0.495*	-0.483*	0.010
Smoking habits	0.535**	-0.360*	-0.139
B-Pb (µg/L)	0.664**	-0.653**	-0.171
B-Cd (µg/L)	0.600**	-0.192	0.126
B-Cr(µg/L)	0.490*	-0.402*	0.063
B-Ni (µg/L)	0.020	-0.092	-0.032
B-Mn (µg/L)	-0.138	0.301	0.009
B-Se (µg/L)	-0.434*	-	0.453
Composite exposure index	0.724**	-0.434*	-0.007

** Correlation significant at p < 0.05, ** correlation significant at p < 0.01, [a] spearman, [b] person correlation coefficient*

The multiple linear regression was applied to estimate the influence of duration of employment, smoking habits, studied blood metals and composite exposure index as independent variable on the frequency of DNA fragmentation as dependent variable among exposed workers. The result shows that the composite exposure index highly affect the frequency of DNA fragmentation with coefficient of determination (R^2 = 0.524, P = 0.0001), table 6.

Table 7 shows that B-Pb was the only variable affect the level of B-Se with coefficient of determination (R^2 = 0.426, P = 0.0001) among the exposed workers.

Table 6: Multivariate regression analysis to identify predictor for DNA fragmentation frequency among exposed workers (n= 26)

dependent variable	Independent variable	β coefficient	t-test	P-value
DNA fragmentation(R^2=0.524) (P<0.0001)	Employment (years)	0.047	0.249	0.806
	Smoking habits	0.057	0.284	0.727
	B-Cd (µg/L)	0.355	0.296	0.572
	B-Pb(µg/L)	0.167	0.609	0.549
	B-Cr (µg/L)	-0.101	-0.474	0.640
	B-Ni (µg/L)	-0.218	-1.542	0.137
	B-Mn(µg/L)	-0.074	-0.513	0.613
	B-Se (µg/L)	-0.054	-0.316	0.755
	Composite exposure index	**0.724**	**5.14**	**0.0001**

Table 7: Multivariate regression analysis to identify predictor B-Se level among exposed workers (n= 26)

dependent variable	Independent variable	β coefficient	t-test	P-value
B-Se (R^2=0.426) (P<0.0001)	Employment (years)	-0.230	-1.339	0.194
	Smoking habits	0.262	1.247	0.225
	B-Cd (μg/L)	0.131	0.746	0.463
	B-Pb(μg/L)	**-0.653**	**-4.219**	**0.0001**
	B-Cr (μg/L)	-0.105	-0.584	0.565
	B-Ni (μg/L)	0.061	0.375	0.711
	B-Mn(μg/L)	0.165	1.042	0.308
	Composite exposure index	0.039	0.127	0.900

DISCUSSION

Foundry particles from different process are consist of a complex chemical mixture which includes Pb, Cd, Ni, Cr and other heavy metals [28]–[30]. Although industrial foundries vary in terms of the type of raw materials used, molten metal being poured, the sand formation, the type of furnace and finishing procedures, the basic process and hazards including particles and metals remain the most important occupational hazards in the foundry industry [2].

In the present work, the exposed workers were deal with different kind of metals during molting and casting at same time, which included cadmium, lead and others.

The measurement of lead level in blood is more suitable and commonly used for medical evaluation of lead exposure [31]**,** moreover it was found a positive correlation between airborne lead and blood lead levels [32]. The monitoring of some metals among the subjects in this study, show significantly higher for B-Pb and B-Cd levels among the exposed workers than in the control participants (P = 0.006). This finding agrees with previously published literatures [7], [32]–[34]. It is clear from the present data that increasing of blood levels of workers who carried out activities in molten and casting sector may be due to the exposure to high levels of airborne lead and cadmium in the workplace, because the main pathway for raising blood lead and cadmium levels among foundry workers is probably inhalation route. This is supported by Chuang et al.[35]. In addition, the exposed workers in the present study did not use any of personal protective equipment (PPE), it is well known that self-protection is important, because the risk of exposure to hazardous materials can be reduced by implementation of appropriate behaviors (i.e. using PPE). In addition, Poor hygiene behaviors may therefore be associated with elevated blood metal levels. Lead and cadmium contaminations could also occur by direct hand-to-mouth contact, and indirectly through contamination of hands, clothes, work surface, drinking water, cigarettes, etc[32]. Ask in and Volkmann[36] reported the effects of personal hygiene on the blood lead levels of workers at a lead-processing facility. Thus, additional reasons for elevating blood metal levels in the workplace are inappropriate behaviors for example, hand-mouth contact with rare hand-washing before eating and drinking.

Blood selenium levels in the present study showed significantly decreased in exposed workers than in the control group (P = 0.017). There was also a significant negative correlation between the B-Pb and B-Se level (P = 0.0001), moreover, inverse relationship between the levels of B-Pb and B-Se (β = -0.653, P = 0.0001) were found. Although there is a paucity of studies assessing the association between B-Pb and Se in lead occupationally exposed subjects, our findings are in agreement with most of the published literature. An inverse relationship between the levels of Pb and Se was also reported in other studies. For example, in a study by Kasperczyk et al.[14] a lower level of Se was reported in Pb-exposed workers compared with that in unexposed controls. Similarly, Gustafson et al.[37] showed significantly lower plasma Se levels in Pb smelters than in the control group. Pawlas et al. [38] found a significant negative correlation between the B-Pb and plasma Se level in workers occupationally exposed to lead. Additionally, the negative correlations between the levels of Pb and Se were also shown in children from urban areas in Poland[39], [40] and Senegal [41]. The above-mentioned associations between Pb and Se may be a result of the formation of inactive Pb-Se complexes and alterations of the absorption and tissue distribution of Pb by Se[19], [39]. There is another mechanism to interpret the decreasing levels of B-Se and its association with B-Pb in workers who have high levels of B-Pb by reducing of oxidative stress through the antioxidant properties of Se[38].

Cytogenetic bio-monitoring studies such as chromosomal aberrations have been proposed as tools to analyze the possible genotoxic effect of a toxicants exposure [42], and considered a relevant biomarker for cancer predisposition [43]. It manifest as chromosomal gaps, chromosomal breaks and dicentric chromosomes [44].

In this study, we investigated the frequencies of chromosomal aberrations in peripheral blood lymphocytes of the exposed workers and control subjects. The difference in prevalence of chromosomal aberrations (CAs) was not statistically significant difference between the two groups (P >0.05), however, the exposed group showed higher frequency in CAs (6, 23.08%) as compared to control one. It is well known that lead and cadmium-induced

genotoxicity due to their ability to interfere with DNA repair processes, both metals could inhibit DNA polymerase β by competition with zinc ions, which are essential in DNA polymerases[45].Moreover, they could inhibit some proteins involved in base excision repairer (BER) that contain zinc-finger motifs, for example Xero derma pigmen to sum group A complementing (XPAC) protein has been shown to be essential for the excision repair process [46]. Furthermore, cadmium have the ability to bind to sulfhydryl groups of glutathione blocks its function as a free radical scavenger[47], lead could inactivate enzymes like glutathione reductase (GR) and/or glutathione peroxidase (GPx), which further depresses the glutathione levels[48], [49] Thus, free radicals become available to cause DNA damage. This mechanism lead to double strand breaks that can be visualized as chromosomal alterations such as breaks and fragments[50].

In the present study, CAs were presented only in workers, who employed in the foundry plant for more 20 years and B-Pb and B-Cd levels were more than (160 and 2.10 µg /L respectively). (i.e high B-Pb and B-Cd levels for long time).Hartwig,[51] reported that lead is weakly mutagenic and can induce DNA strand breaks in cells only when incubated for long time with a high doses.

Several studies found a marked increase in the frequencies of chromosomal aberrations in human peripheral leucocytes of workers occupationally exposed to cadmium as compared to controls [52]–[54]. We could explain this discrepancy in the results obtained by various studies on the fact that the workers exposed to different metal levels during their activities and the differences in concentrations at target sites on one hand, or to the capacity of the body to eliminate or repair injured cells in vivo on the other.

Apoptosis is characterized with a number of crucial cellular events, one of these is genomic disassembly and the cleavage into oligonucleosomal fragments[55]. In order to investigate DNA fragmentations during apoptotic cell death, we carried out the DNA fragmentation assay by agarose gel electrophoresis of DNA isolated from blood of controls as well as from exposed workers.

In the present study, the exposed group unveiled a significantly higher frequency of DNA fragmentation (8, 30.77%), (P = 0.0001). Also there were significant positive association between DNA damage with duration of employment, smoking habits, B-Pb, B-Cd, B-Cr and composite exposure index, furthermore the composite exposure index was the only predictor to DNA fragmentation, which reflect that metals may act synergistically to induce DNA fragments. Despite the fact that a detailed molecular mechanism by which co-exposure to lead, cadmium and chromium accelerates DNA damage is still not understood, and depend on the fact that these metals can induce oxidative stress via producing of reactive oxygen species in exposed workers by different mechanisms[56], might suggest the involvement of oxidative stress. Since reactive oxygen species are highly reactive with DNA and subsequently can induce apoptosis by up regulation of proapoptotic proteins and down regulation of anti-apoptotic proteins [57].

The measured changes in DNA fragmentation due to oxidative stress could depend on a variety of parameters that affect the interpretation of data. These parameters concern the steady-state between mature and new-born WBCs, changes in endonuclease and protease activities andDNA repair, the contribution of dying or dead (apoptosis or necrosis) WBCs, and diet[58].

Previous study conducted by Olewińska et al.[59] showed that the agarose gel electrophoresis revealed the DNA fragmentation pattern in humans exposed to lead, also, DNA fragmentation observed herein by gel electrophoresis was observed by Moniem et al.[60] in rats and Ahmed et al.[61]in female rabbits treated with lead.

Regarding the impact of lead and cadmium on hematological parameters, a significantly lower hemoglobin concentration was found among exposed workers versus the controls (P = 0.012). But we did not observe any significant change in the other parameters namely WBCs and RBCs (P = 0.241; 0.791 respectively).

It was reported that Lead has multiple hematological effects, such as; changed red blood cell morphology and shortened its life span due to the increased mechanical fragility of the cell membrane, as well as, inhibited of hemoglobin synthesis[62], [63]. In heme synthesis there are several enzymes catalyzing the heme biosynthesis pathway, one of them δ-aminolevulinic acid dehydratase (ALAD), which possessing sulfhydryl groups. Lead and cadmium inhibit the activity of ALAD due to the binding affinity of these metals to thiol groups[64], [65].

Since, Pb and Cd are widely used in recent years around the world, co-exposure pose a major challenges for international agencies which included the data gaps, and poorly understood pathways for their chemical interactions. This mixture may induce bioactivation sites resulting in the increase of their toxicity which observed as markedly decreased of B-Se level and this effect accompanied with increased of DNA fragmentation patterns and CAs as well

as significant reduction in hemoglobin levels in foundry workers. So, health and safety education for workers by explaining hazards and their protective measures, as well as, training workers on the use of emergency measures are recommended.

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