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Original Article

The association of occupational metals exposure and oxidative damage, telomere shortening in fitness equipments manufacturing workers

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Abstract: The welding is the major working process in fitness equipment manufacturing industry, and International Agency for Research on Cancer has classified welding fumes as possibly carcinogenic to humans (Group 2B). The present study aimed to evaluate associations between the occupational exposure of metals and oxidative damage and telomere length shortening in workers involved in the manufacture of fitness equipment. The blood metal concentrations were monitored and malondialdehyde (MDA), alkaline Comet assay was determined as oxidative damage in 117 workers from two representative fitness equipment manufacturing plants. MDA levels varied according to workers' roles at the manufacturing plants, and showed a trend as cutting > painting > welding > administration workers. Welders had marginally shorter average telomere lengths than the administrative workers ($p = 0.058$). Cr and Mn levels were significantly greater in welders than they were in administrative workers. There were significantly positive correlations between MDA and Cr and Mn levels, the major components of welding fume. However, the association would be eliminated if co-metals exposure were considered simultaneously. In future, telomere length and MDA might be potential biomarkers for predicting cardiovascular disease in co-metals exposed workers.

Key words: Telomere length, Welding, Metals, Oxidative damage, MDA

Introduction

Many toxic substances, including heavy metals, ozone, carbon monoxide, carbon dioxide, and nitrogen oxides, are generated during welding[1]. Since 1991, welding fumes have been recognised by the international agency for research on cancer (IARC) as class 2B carcinogens. Previous studies have shown that heavy metals, including Cd, Mn, Cr, and Fe, all of which have been shown to be related to the generation of reactive oxygen species (ROS), are major components of welding particles[2–4]. Increased production of ROS can trigger other signals that further increase oxidative stress and lead to disease. The oxidative stress exerts a greater influence on telomere shortening with end-replication because GGG triplets in the telomere have been shown to be sensitive to hydroxyl radicals[5].

The three main causes of telomere length shortening are first, the inability of DNA polymerase to replicate a liner DNA molecule to its ends; second, unrepaired oxidation damage[6]; and third, the action of a C-strand-specific exo-

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nuclease[7]. Oxidative stress can accelerate telomere shortening by two processes: by increasing telomere losses in hematopoietic stem cells, and by diminishing the biological lifetime of peripheral leukocytes[6]. Reactive oxygen species (ROS) and hydroxyl radicals in particular produce single-strand breaks, either directly or as an intermediate step in the repair of oxidation base modifications[8]. Oxidative damage is repaired less well in telomeric DNA than it is in the chromosomes[9]; thus, oxidative stress plays a greater role in the acceleration of telomere loss. After alkylation, single-strand breaks accumulate randomly along the telomere; thus, the telomere-shortening rate can be increased or reduced by modifying the amount of oxidative stress[6]. A continuous exponential correlation between cellular oxidative stress levels and the telomere-shortening rate was found in fibroblasts[10]. Von Zglinicki *et al.*[9] suggested that oxidative stress is an important factor in telomere shortening, and that telomere-driven replicative senescence is a primary stress response that may have evolved to block cell growth and reduce the risk of mutation formation. The measurement of telomere length, a biomarker of chronic oxidative stress, has been considered[8]. In occupational studies of coke oven workers, telomere shortening was found to be associated with chronic polycyclic aromatic hydrocarbon (PAH) exposure[11], and with exposure to high levels of traffic pollutants, including benzene and toluene in traffic police[12]. The present study aimed to evaluate associations between the occupational exposure of metals and oxidative damage and telomere length shortening in workers involved in the manufacture of fitness equipment. We also assessed the risk of adverse health effects arising from the workers' telomere shortening and oxidative stresses.

Methods

Subjects selection

The study was conducted at two typical fitness equipment-manufacturing plants in Central Taiwan. A pre-sampling walkthrough was conducted to determine the layout of each site and its borders. The main production operations include steel cutting, manual or auto metal arc welding, painting, assembly, installation of integrated circuitry, and equipment testing. The zones assigned to the various stages in the manufacturing process were based on workflow and function.

The ethics approval was approved by the Ethics Committee of Chung Shan Medical University (Taichung, Taiwan) and all participants signed the informed consent prior to join this study. In the beginning, seventy-five manufacturing workers and forty-nine administrative workers were recruited from 2 plants. Each employee was asked to fill out a questionnaire asking for information about personal characteristics (gender, age, height, weight, residence neighborhood, etc.), life style (e.g., tobacco usage and alcohol intake), and occupational history (e.g., working history at current place of employment, working environment, job titles, periods of employment, and use of protective equipment). Overall, 124 workers were recruited in this study, and 117 belong to cutting department (N=2), painting department (N=6), welding department (N=60), and administrative department (N=49). Seven workers situated in manufacturing department at the time of the study period, however, their job involved quality assurance or quality control (QA/QC) or just for computer drawing. Therefore, they cannot be well grouped into exposure or control group, and their analyzed data were excluded in the next analysis. Finally, the results of metal analysis of 8 welders and 2 administrators cannot met the criteria of our QA/QC, then the data were also deleted in this study.

Blood collection

The sampling procedure was processing at Friday, the final of a weekday. Friday would indicate the hazards accumulation within a week. The workers need to complete an overnight fast for drawing blood. Each participant was drawn 6 ml of venous blood into chemically clean tubes containing heparin and EDTA. One ml blood sample was centrifuged at $1,000 \times g$ for 10 min for separating blood cells and plasma. A ml cryoprotectants (antifreeze compounds, mixed by RPMI 1,640 power, $NaHCO_3$, FBS) was used as anti-freezer to add to 1 ml whole blood, and then the whole blood and plasma was stored at −85°C until the comet assay and MDA analysis. The other whole blood samples with heparin were stored at −20°C until metals assay.

Blood metal analysis

Two milliliters of venous blood from each worker was stored in a tube containing heparin. One milliliter of the blood sample was centrifuged at 1,000 g for 10 min to separate blood cells from plasma. The 500 μl serum were diluted to 5 ml with 5% ultrapure nitric acid (HNO_3, analytical grade, Merck), and 250 μl 30% H_2O_2 (Sigma). Inductively coupled plasma mass spectroscopy (ICP-MS, Perkin Elmer Sciex ELAN DRCII) was used for analyzing the metal content. In this study, the recovery efficiency tests were conducted using the same sample analysis pro-

cedure with the addition of a standard solution. All of 60 manufacturing workers and 47 administrators completed the blood metal analysis.

For the determination of Cr, Fe, Co, Cu, Zn, Mn, Cd, Mg, and As contents, an internal standard (100 μl of 10 μg/L Rh, Fluka Int.) was added to the filtered solution for quality control. The accuracies of the analytical method and instrumentation were validated using certified reference materials from the Perkin Elmer Pure Elan 6100 DRC (Lot#6-218). In this study, the recovery efficiency tests for Cr, Fe, Co, Cu, Zn, Mn, Cd, Mg, and As were conducted using the same sample analysis procedure with the addition of a standard solution prior to extraction.

MDA analysis

MDA levels was measured to represent the plasma lipid peroxidation. The MDA assay protocol was the same as the previous study from Liu *et al.* [13]. The MDA standard curve were in concentrations 0.075 to 1.0 μM of 1,1,3,3-tetraethoxypropane (Fluka Co., No. 87670). At first, 50 μl of 0.2% butylated hydroxytoluene (BHT) and 25 μl 10 N NaOH was added to 500 μl of plasma. Then the mixture combined with 30 ml of 1% potassium iodide (KI) and 7.2% trichloroacetic acid (TCA) was incubated at 60°C for 30 min. After the mixture were centrifuged at 2,300 × *g* for 10 min, and then 1.0 ml of 0.6% thiobarbituric acid (TBA) was added to the mixture and incubated at 95°C for 30 min. Finally, the samples were placed in ice for 5 min, and then 3.0 ml of n-butanol was added. The n-butanol extract was measured by a fluorescence spectrophotometer (Shimadzu RF-5301pc) with an excitation wavelength of 515 nm and an emission wavelength of 555 nm. Finally, 68 manufacturing workers and 49 administrators completed the analysis of oxidative damages.

Blood comet assay for DNA strand breakage

The comet assay protocol was adopted from our previous report[14]. In the current study, we didn't use the formamido pyrimidine glycosylase (Fpg) to verify specific 8-oxodG in the comet assay. At first, 150 μl of 1% normal melting point agarose (LMA) was applied on the first layer of the slide. Then 50 μl of whole blood with 250 μl of 1% LMA was mixed, and 130 μl mixture was applied to be the second layer of the slide. After 150 μl 1% NMA was applied to be the third layer, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris, 1% *N*-sodium lauroyl sarcosinate) for 1 hr at 4°C. The slides were then placed in an electrophoresis tank for 15 min to let DNA to unwind in the alkaline solution (300 mM NaOH and 1 mM Na_2 EDTA). Electrophoresis was carried out at 300 mA for 20 min in alkaline solution at room temperature. The slides were further neutralized by adding 0.4 M Tris-HCl buffer (pH 7.5) and stained with ethidium bromide. Images of DNA strand breakage were visually analyzed based on comet scores as the previous report[13]. The tail moment of DNA was measured by using Comet Assay IV (Perceptive Instruments Ltd., Haverhill, Suffolk, UK) according to the formula:

Tail moment of DNA = TDNA (DNA in tail as a % of total DNA) × TDx (DNA tail length).

The break of DNA strand may be related to the oxidative damage and a comet was formed by electrophoresis. The longer DNA tail length and the higher percentage of breakage in tail of DNA would appear due to the elevated damage. The tail moment is regarded as one of the best indices of comet formation obtained in computerized analysis[15].

Telomere length measurement

DNA was extracted from whole-blood lymphocytes using a DNA extraction kit (Cat.: GC2002, Lot: JoA10-JAH10,VIOGENE Inc.) and following standard procedures. We used southern blotting analysis to detect the locations and intensities of radioactive signals. Telomere length was determined using a Telo TAGGG Telomere Length Assay Kit (Roche Diagnostics GmbH, Mat. No: 12209136001). At first, the purified genomic DNA was digested by an optimized mixture of frequently cutting restriction enzymes. The enzymes were designed based on telomeric DNA and sub-telomeric DNA could not be cut. Non-telomeric DNA was digested to low molecular-weight fragments. After DNA digestion, the DNA fragments were separated by gel electrophoresis, then those were transferred to a nylon membrane by Southern blotting. The blotted DNA fragments were hybridized to a digoxigenin (DIG)-labeled probe, specific for telomeric repeats, then incubated with a DIG-specific antibody covalently coupled to alkaline phosphatase. Finally, chemiluminescence signals were scanned using an imaging system to quantitatively determine the average terminal restriction fragment lengths by comparing the length of a specific smear to a molecular standard.

Statistical methods

The JMP 5.0 (SAS Institute, Cary, NC, USA) software packages were used for data management and statistical analysis. The Wilcoxon rank sum test was carried out to evaluate difference in telomere length and MDA levels, as well as DNA strand breakage between welding and

administrative workers. Differences between the gender ratios, smoking ratios, and other factors were examined using Fisher's exact test. The correlations between oxidative damages, telomere length and blood metal levels were tested by Spearmen test. Moreover, multiple regression analysis was also used to assess the associations between occupational exposure, and oxidative damages after adjustment for age. The metals those put into the regression model were selected based on the results of correlations between blood metals and oxidative damages. The selection criteria were set at p value lower than 0.2. The statistical significant was set at p value <0.05.

Results

Table 1 shows an outline of the demographic characteristics of the manufacturing and administration workers involved in this study. More workers were in the manufacturing department than in administration. Additionally, more smokers and alcohol drinkers worked in the manufacturing departments than those who worked in administration. A greater percentage of workers in the manufacturing departments were at risk of exposure to particulates, and thus, used respirators, compared to those in the administrative department.

Biological effect markers

Workers were categorized based on their job role and work departments, as members of cutting (n=2), welding (n=60), painting (n=6) or administration workers (n=49). Table 2 shows that the tail moments of DNA breaks in painting workers (9.63), cutting workers (6.26), and welding workers (6.17) are greater than those of administration workers (5.20), and that there is a signifi-

Table 1. Demographic characteristics of the study subjects

	Manufacturing dep. (n=75)	Administrative dep. (n=49)	p value
Sex[b]			
Male	61 (81.3)	14 (28.6)	**<0.001***
Female	14 (18.7)	35 (71.4)	
Age (Year)[a]	34.0 ± 10.0	32.7 ± 6.9	0.448
BMI[a]	23.4 ± 5.3	22.3 ± 3.8	0.251
Body weight (kg)[a]	62.6 ± 15.5	60.8 ± 14.7	0.177
Waistline (cm)[a]	78.9 ± 10.6	74.8 ± 10.0	**0.031***
Working year[a]	3.5 ± 3.9	3.4 ± 3.5	0.941
Smoking status[b]			
Yes	32 (42.7)	2 (4.1)	**<0.001***
No	43 (57.3)	47 (95.9)	
Alcohol drinking[b]			
Yes	10 (13.3)	1 (2.0)	**0.049***
No	65 (86.7)	48 (98.0)	
Use of betel nut[b]			
Yes	5 (6.7)	0	0.156
No	70 (93.3)	49 (100)	
Excise regular[b]			
Yes	27 (36.0)	23 (46.9)	0.263
No	48 (64.0)	26 (53.1)	
Particle exposure[b]			
Yes	65 (86.7)	2 (4.1)	**<0.001***
No	10 (13.3)	47 (95.9)	
Use of respirators[b]			
Yes	69 (92.0)	12 (24.5)	**<0.001***
No	6 (8.0)	37 (75.5)	

[a] mean ± SD, by students' test

[b] number with percentage in parentheses, by Chi-square test

*$p<0.05$

Table 2. Distribution of malondialdehyde (MDA), tail moment, telomere length among the workers in cutting, painting, welding and administrative departments

	Cutting (n=2)	Painting (n=6)	Welding (n=60)	Administrative (n=49)	p value
MDA (μM)	5.0 ± 0.13	4.36 ± 0.35	3.98 ± 0.86	3.56 ± 1.16	0.099
tail moment	6.26	9.63 ± 4.31	6.17 ± 3.61	5.20 ± 2.97	0.029*
telomere length (kbp)	15.7 ± 0.71	8.70 ± 0.84	10.07 ± 3.13	11.65 ± 4.03	0.058

mean ± SD, except for the limited sample of cutting department, the other 3 groups were tested by Kruskal-Walllis

*$p<0.05$

Table 3. The correlations among the biomarkers of oxidative damage and telomere length in the workers of welding departments

r	MDA	Telomere length
Tail moment	−0.003 (0.973)*	−0.133 (0.141)
MDA	—	−0.080 (0.378)

*correlation coefficient and p value inside parenthesis by Spearman's test

Table 4. The differences of blood metals concentrations of workers between welders and administrative workers

	Cutting dep. (n=2)	Painting dep. (n=6)	Welding dep. (n=52)	Administrative dep. (n=47)	*p* value
Cr (μg/L)	4.50	2.29 ± 0.60	2.60 ± 2.12	2.27 ± 3.36	**0.004***
Fe (mg/L)	584.3	553.5 ± 51.3	506.3 ± 120.8	891.3 ± 202.6	**<0.001***
Co (μg/L)	17.16	14.96 ± 1.65	16.23 ± 1.85	17.17 ± 5.67	0.521
Cu (μg/L)	622.8	576.3 ± 33.4	608.2 ± 170.6	894.7 ± 193.5	**<0.001***
Zn (mg/L)	5.80	5.34 ± 0.50	4.89 ± 1.06	6.64 ± 1.55	**<0.001***
Mn (μg/L)	26.46	17.82 ± 2.53	16.55 ± 7.45	14.0 ± 10.13	**0.012***
Cd (μg/L)	0.71	0.56 ± 0.50	0.87 ± 1.50	2.70 ± 3.86	**0.004***
Mg (mg/L)	44.10	37.72 ± 2.88	36.10 ± 6.70	59.63 ± 9.49	**<0.001***
As (μg/L)	11.53	11.65 ± 2.02	9.05 ± 2.12	12.20 ± 4.89	**<0.001***

*$p<0.05$

mean ± SD, except for the limited sample of cutting and painting departments, the other 2 groups were tested by Wilcoxon test

cant difference between welding workers and administrative workers ($p=0.029$). However, there are no significant differences between painting and welding ($p=0.640$) and between painting and administration workers ($p=0.132$) by Wilcoxon test. The levels of MDA in workers showed a decreasing trend in the order cutting > painting > welding > administration workers, there was only a marginally significant difference between welding and administration workers ($p=0.099$). Average telomere lengths were 15.7, 8.70, 10.1, and 11.65 kbp in cutting, painting, welding, and administration workers, respectively. Welders had marginally shorter telomere lengths than the administrative workers ($p=0.058$). There were no significant correlations between DNA break tail moment, MDA levels, and telomere length among the welding workers (Table 3).

Blood metal content

The concentrations of blood Fe, Cu, Zn, Cd, Mg, and As were significantly greater in administrative workers than they were in welders, and Cr and Mn levels were not significantly different (Table 4).

Blood metals and biomarkers

Table 5 shows correlations between biological effect markers and metal ion concentrations. The table shows significantly positive correlations between MDA and Cr and Mn levels, the major components of welding emissions. Telomere length has significantly negative correlations with blood Cr, Co, and As levels. Table 6 shows the results of multiple regression analysis between MDA levels, telomere length, and blood metal levels of the welding workers. There are significant negative relationships between telomere length and age, levels of As ($r=-0.335$ and -0.411, $p=0.020$ and 0.031 for age and As, respectively).

Table 5. The correlations of blood metals levels and oxidative damages, and telomere length of welding departments (n=60)

	Tail moment	MDA	Telomere length
Cr (μg/L)	0.171 (0.226)	**0.306 (0.028)***	**−0.279 (0.046)***
Fe (mg/L)	−0.006 (0.966)	0.190 (0.178)	0.046 (0.748)
Co (μg/L)	−0.001 (0.997)	−0.101 (0.476)	**−0.247 (0.078)**
Cu (μg/L)	0.024 (0.866)	0.036 (0.798)	−0.157 (0.267)
Zn (mg/L)	0.071 (0.619)	0.118 (0.405)	−0.216 (0.125)
Mn (μg/L)	−0.008 (0.955)	**0.317 (0.022)***	0.019 (0.896)
Cd (μg/L)	**0.302 (0.029)***	0.178 (0.207)	−0.186 (0.187)
Mg (mg/L)	0.112 (0.429)	0.075 (0.599)	0.184 (0.191)
As (μg/L)	0.048 (0.735)	−0.067 (0.639)	**−0.299 (0.033)***

*$p<0.05$

tested by Spearman correlation

Table 6. Multiple regression analysis between telomere length, MDA levels, and blood metals concentrations of the workers in industry of manufacturing fitness equipment

Dependent variable	Independent variables	R-square	Regression coefficient	*p* value
MDA#		0.154		0.019*
	Intercept		1.035	0.004*
	Age#		0.410	0.072
	Smoker		0.008	0.755
	Cr#		0.003	0.970
	Mn#		0.343	0.141
telomere length#		0.150		0.040*
	Intercept		1.964	<0.001*
	Age#		−0.335	0.020*
	Smoker		−0.005	0.754
	Cr#		0.002	0.981
	Mn#		0.111	0.451
	Co#		−0.169	0.682
	As#		−0.411	0.031*

#: The data has been logarithmic transformed *$p<0.05$

The metals those put into the regression model were selected based on the results of correlations between blood metals and oxidative damages (Table 5). The selection criteria were set at *p* value lower than 0.2.

Meanwhile, the marginally positive association was only found between MDA levels and blood Mn content after adjustment for age and smoking status.

Discussions

Oxidative stress in workers of manufacturing fitness equipment

The analysis of telomere length is a new technology apply on occupational studies or environmental exposure groups. For example, Pb exposure in childhood appeared to be associated with shorter telomeres[16].; Telomere length shorten in tobacco farmers was influenced by exposure to pesticides[17].; The shorter sperm telomere length was found to be associated with polycyclic aromatic hydrocarbon (PAHs) exposure and sperm telomere length seemed to be a potential biomarkers to predict the effects of low-level exposure to PAHs on human sperm[18].; The telomere length might be considered as a potential biomarker for response the toxicity of welding fumes those contains genotoxic (e.g., Cr, Ni) and soluble (e.g, Cr, Mn) metals[19]. All of the above studies have mentioned that telomere length could be a potential biomarker in predicting occupational exposure or environmental on oxidative damage.

The tail moment of DNA breaks was greatest in painting workers, followed by cutting and welding workers; administrative workers were least affected. Meanwhile, the trends in MDA level and telomere length were inconsistent with DNA breaks among the four work categories (Tables 2 and 3). From the literatures review, the mean telomere length of never smokers at age 79 were 6.75 kbp[20, 21]; the average level were 7.06 kbp at age 50.65 in healthy women[22]; in people aged 60 yr or older, their telomere length ranged from 1,930 to 4,310 bp[23]; In 231 Japanese male subjects aged 20–68 yr, their telomere length were 23.1–9.4 kbp[24]. Though some of studies showed the shorter telomere length than our finding (the average telomere length was 10.07 kb in welders), the reason was attributable to the older of the subjects. The manufacturing workers experience greater oxidation effects than do administrative workers. An *in vivo* study showed that acute exposure of rats to welding fumes caused noticeable oxidative damage[25] and lipid peroxidation[25, 26]. However, DNA damage caused by long and repeated exposure to welding fumes may be repaired[26], and this may explain why MDA levels did not appear to correlate with the numbers of DNA strand breaks. This may be the reason that the DNA break is not in correlated with their blood metal levels in welders.

Metals in welding fumes

The predominant metals found in welding fume were Cr[27], Fe, Mn, and Ni[28]; stainless steel is composed of Fe, Cr, Mn, and Ni[29]. The greater Cr, Pb, and Ni concentrations were found in blood and urine samples of welders than were detected in control workers[30], and Botta *et al.*[31] found greater concentrations of Cd, Co, Cr, Ni, and Pb in the blood of welders compared to in the blood of the control group. Berlinger *et al.*[32] reported that the water-soluble metal components Fe, Mn, Cr, Ni, and Cr (VI) in the respirable aerosol fraction were 60%–97% of inhalable aerosols, and 64%–94% of total metal concentration. Collectively, these studies suggest that Cr and Ni are the main metals emitted during the welding process. However, in the present study, the Ni concentrations were determined at less than half of the limit of detection (LOD). With the exception of Cr and Mn, metal content showed greater concentrations in the blood of administrative workers than in the blood of welders. Mn levels in the blood of welders (16.6 μg/L) and administrative workers (14.0 μg/L) in the present study were greater than those found in factory workers (5.5 μg/L), a control group in Taiwan (5.4 μg/L)[33], and South Korean adults (10.8 μg/L)[34]. Our findings indicated that administration workers may expose to welding fumes by leakage through the building because the offices floor were sited within the same building. Although manufacturing workers showed lower blood As levels (9.1 μg/L) than the administrative workers did, the evidence still showed the oxidative damage in this kind of workers.

Metal exposure and oxidative damage

For male manufacturing workers, the Cd and As content were slightly higher than those in administrators. For non-smokers, no significantly differences of Cd and As levels were found between administrators and manufacturing workers. To get the real association between metal exposure and oxidative damage, the current study only took the data obtained from the welders for further correlation analysis. In the beginning of the multiple regression, the parameters about hypertension and diabetes were considered as independent variables simultaneously, however, the regression model were not qualify (R-squared = 0.324 and $p = 0.588$). Therefore, Table 6 showed the final model that presented the significant association between telomere length and As exposure by adjusting the factor included age[20, 35] and smoking status[36] that might affect the telomere length.

A concentration-dependent increase in DNA damage and lung macrophage death was found after *in vitro* treat-

ment with a stainless steel sample, which with abundant metals, and the number of apoptotic cells in lung tissue was increased after *in vivo* welding fume treatment[25]. The urinary Cr levels in welders was found to exceed the biologic exposure index limit, and exhibited increased plasma lipid peroxidation and lipid susceptibility to oxidation, compared to matched controls[37]. Welders' increased exposure to Cr and Ni resulted in an exposure-dependent increase in single-strand DNA breakages and elevated sister chromatid exchange[38], the reason was that welding fumes from stainless steel welding could produce biologically reactive hydroxyl radicals (•OH) from the reduction of Cr(VI) to Cr(V). These studies could explain our observation that Cr exposure positively correlates with the MDA level. Wang *et al.*[4] suggested a possible mechanism for Cd-induced generation of ROS in the mitochondria and induced oxidative damage in cells; in the present study, we found a positive association between DNA strand breakages and blood Cd levels in welders.

Residents living along the northeast coast of Taiwan are at risk of ingesting As-contaminated water, which might cause adverse effects by increasing their exposure to reactive oxidants and reducing their antioxidant capacity[39]. An epidemiological study showed significantly greater 8-OH-dG levels, a DNA marker for oxidative stress[40] and increased higher lipid peroxide (LPO) production[41] in an As exposed population compared to a low-exposure group. These reports suggest that As causes oxidative damage and further plays a role in the development of cancer[42]. Although As levels seen in our study were less than those reported form the occupational group in Taiwan[33], this is the first study to relate telomere shortening with As exposure. Flora[43] identified that As suppresses cell cycle checkpoints and induces cell cycle arrest. Telomere shortening might be a trigger for p53-dependent cell cycle arrest[44]. Exposure to As induces a significant decrease in antioxidant agents within the body, and increases generation of free radical[39, 43]. The subsequent rise in MDA levels, can result to increased DNA fragmentation[43, 45]. DNA strand breaks can be formed either directly or as an intermediate step if telomeric DNA fails to repair single-strand breaks. Additionally, oxidative stress induced telomere shortening can occur because of the unrepaired nucleotides, or base damage might interfere with the replication fork[8]. Furumoto *et al.*[35] identified that reductions in oxidative stresses generated by antioxidant treatments might reduce the telomere shortening and delay cellular senescence. The limitation of the present study is that the limit workers in each working zones, and due to the small sample size, future occupational epidemiological study should be considered in discussing how we could use telomere length and MDA markers to make predictions for cardiovascular disease in large population with co- metals exposure. Meanwhile, blood ROS can be affected by hepatitis, COPD or other confounding factors such as inflammation, thus exposure index should be analyzed with controlling these confounding factors in future study design.

Conclusion

The concentrations of blood Cr and Mn were significantly greater in welders than they were in administrative workers. The tail moments of DNA breaks showed a significant difference between welding workers and administrative workers. The significantly positive correlations were only found between MDA and Cr and Mn levels, the major components of welding emissions. However, the association would be eliminated if co-metals exposure were considered simultaneously. In future, telomere length and MDA might be as potential biomarkers for predicting cardiovascular disease in co-metals exposed workers due to telomere length seems to be suggested as a biomarker for chronic oxidative stress.

Competing Interest

All authors declared no conflicts of interest.

Authors' Contributions

Chen and Ko designed the study and applied for Research Ethics Board approval. Chen and Cheng recruited the participants and Liu collected the data. Hsin and Liu completed all analysis, and further Chen and Ko analyzed the data and prepared draft figures and tables. Chen prepared the manuscript draft with important intellectual input from Ko and Liu. All authors approved the final manuscript. Chen, Ko and Liu had complete access to the study data.

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