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Lipid Peroxidation and Oxidative Status Compared in Workers at a Bottom Ash Recovery Plant and Fly Ash Treatment Plants

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Abstract: Lipid Peroxidation and Oxidative Status Compared in Workers at a Bottom Ash Recovery Plant and Fly Ash Treatment Plants: Hung-Shin LIU, *et al*. Department of Occupational Safety and Health, Chung Shan Medical University, Taiwan—Fly ash and ambient emissions of municipal solid waste incinerators contain polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), other organic compounds, metals, and gases. Hazardous substances such as PCDD/Fs, mercury vapors and other silicates, and the components of bottom ash and fly ash elevate the oxidative damage. We compared oxidative damage in workers exposed to hazardous substances at a bottom ash recovery plant and 3 fly ash treatment plants in Taiwan by measuring their levels of plasma malondialdehyde (MDA) and urine 8-hydroxydeoxyguanosine (8-OH-dG). Significantly higher MDA levels were found in fly ash treatment plant workers (3.20 μM) than in bottom ash plant workers (0.58 μM). There was a significant association between MDA levels in workers and their working environment, especially in the fly ash treatment plants. Levels of 8-OH-dG varied more widely in bottom ash workers than in fly ash workers. The association between occupational exposure and 8-OH-dG levels may be affected by the life style of the workers. Because more dioxins and metals may leach from fly ash than from bottom ash, fly ash treatment plant workers should, as much as possible, avoid exposing themselves to fly ash.
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Twenty municipal waste incinerators were built and began operating in Taiwan from 1999 to 2005. As a by-product of waste treatment, the incinerators produce residues containing bottom ash and fly ash. Approximately 10–15% of the residues are bottom ash and 2–3% are fly ash which is obtained from different air pollution control devices. Therefore, about 2,250 tons of bottom ash and 400 tons of fly ash are generated daily in Taiwan[1)]. Various environmental hazards and metals are liberated into either bottom ash or carried away with gases which are subsequently trapped in fly ash.

Bottom ash remains to be disposed of and has long been disposed of in landfill facilities. However, studies have found that the bottom ash from the municipal solid waste incinerators is composed of fine ash material and melted components, including small quantities of crystallized metallic components, ceramics, and stones. These combustion residues are more commonly recycled for their metal content rather than being disposed of in landfills[2)].

Rowat[3)] pointed out the lack of adequate disposal techniques for incinerator fly ash; however, fly ash is commonly solidified in Taiwan. Bottom ash utilization in the Netherlands is about 100%, and it requires isolation precautions before use. In the present study, a plant recovering the isolated ferrous and non-ferrous metals from bottom ash was included.

The fly ash and ambient emissions of municipal solid waste incinerators are known to contain polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), other organic compounds, metals, and gases[3–8)]. A leaching study[1)] found that the concentration of lead in fly ash was higher than the regulatory level set by the Taiwan EPA. Thus,

fly ash was considered as a hazardous waste. It is highly likely that incinerator workers are exposed to bottom ash and fly ash when they handle the residues of incinerations. In addition, after they had compared the characteristics of bottom ash and fly ash, Chang and Wey[1] found that most of the particles were probably in the range of 1,680–4,750 μm in bottom ash, and that the particle size of fly ash was notably smaller than 149 μm[1]. Therefore, the toxic effects may be different for workers exposed to the bottom and fly ash due to the different particles penetration into the lungs.

Hazardous substances such as PCDD/Fs, mercury vapors, and other silicates which are components of bottom ash and fly ash, have been shown to increase plasma levels of malondialdehyde (MDA) in exposed people[9–11]. MDA is a biological marker of lipid peroxidation caused by oxidative stress[12, 13], and previous studies have suggested that occupational exposure to incinerators may cause harm via oxidative stress[9–11].

Although a few studies have reported oxidative stress in workers at municipal waste incinerators[10, 14], little is known about the status of oxidative stress in workers at bottom and fly ash treatment plants, who have a greater potential for occupational exposure. In the present study, we compared oxidative damage in workers potentially exposed to hazardous substances, including PCDD/Fs, metals, and silicates, at a bottom ash recovery plant and fly ash treatment plants in Taiwan by measuring their levels of plasma MDA and urine 8-hydroxydeoxyguanosine (8-OH-dG).

Materials and Methods

Participants

One typical bottom ash recovery plant and 3 fly ash treatment plants were included in our study. A bottom ash recovery plant handles the bottom ash from incinerators, and three fly ash treatment plants solidify fly ash. A pre-sampling walk-through was conducted to comprehend the layout of each work site and manufacturing process in these plants. In principle, the two kinds of plants were identified as PCDD/F, metal, and silicate emission sources because they handled the residues of the incineration.

Thirty-seven workers were recruited from the bottom ash recovery plant and forty-one workers from the fly ash treatment plants in Taiwan, all of whom signed a consent form. This study was approved by the Ethics Committee of the Kuang Tein General Hospital (Taichung, Taiwan). The workers were assigned to white-collar or blue-collar groups based on their operational departments or job titles. We used a questionnaire to gather information on personal characteristics (gender, age, height, weight, neighborhood, and others), life style (tobacco usage, alcohol intake, and exercise habits), and occupational history (e.g., working histories at smelting plants, working environment, job titles, periods of employment, and use of protective equipment).

Urine collection

Urine samples of workers were collected in polyethylene bottles from the first urination after the workers began work in the morning. They were kept at –85°C until further analysis.

Blood collection

After completing an overnight fast, each participant provided 1 m*l* of venous blood, drawn into chemically clean tubes that contained heparin. The blood sample taken from each worker was centrifuged at 1,000 × g for 10 min to separate erythrocytes and plasma. Plasma was stored at –85°C until analysis of MDA.

MDA analysis

Plasma lipid peroxidation was measured as the levels of MDA. The MDA standard solution used were 1,1,3,3-tetraethoxypropane (Sigma-Aldrich Co., St Louis, MO) in concentrations of 0.075, 0.1, 0.25, 0.4, 0.5, 0.75, and 1.0 μM. First, 250 μl of plasma was added to 25 μl of 0.2% butylated hydroxytoluene (BHT) and 12.5 μl 10N NaOH. After the mixture had been incubated at 60°C for 30 min, it was combined with 1.5 m*l* of 1% potassium iodine (KI) and 7.2% trichloroacetic acid (TCA). The samples were then centrifuged at 2,300 × g for 10 min. Next, 0.5 m*l* of 0.6% thiobarbituric acid (TBA) was added into the mixture, and incubated at 95°C for 30 min. Finally, samples were placed in ice for 5 min, then 1.5 m*l* of normal (n-)butanol was added. The n-butanol extract was measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm using a fluorescence spectrophotometer.

Urine 8-OH-dG assay

Urinary 8-OH-dG levels were determined using a competitive ELISA immunoassay (Japan Institute for the Control of Aging, Shizuoka, Japan)[15]. Data are presented as 8-OH-dG (μg/g creatinine).

Statistical methods

The JMP 5.0 (SAS Institute, Cary, NC) and Statistica 6.0 (StatSoft, Inc., Tulsa, OK) software packages were used for data management and statistical analysis. The Wilcoxon rank sum test was performed to evaluate differences in the age, working histories, and 8-OH-dG and MDA levels of workers between the two types of plant. Differences between the gender ratios, smoking ratios, and other factors were examined using Fisher's exact test.

Table 1. Demographical characteristics and occupational histories of workers in bottom and fly ash treatment plants

	Bottom ash (n=37)	Fly ash (n=41)
Age (range)[a]	34.8 (19.6–58.1)	35.4 (22.5–56.6)
Gender (%)[b]		
Male	30 (81.1)	38 (92.7)
Female	7 (18.9)	3 (7.3)
Smoking status (%)[b]		
Smokers	17 (45.9)	22 (53.7)
Non-smokers	20 (54.1)	19 (46.3)
Alcohol drinking (%)[b]		
Drinker	15 (40.5)	13 (31.7)
Non-drinker	22 (59.5)	28 (68.3)
Exercise regularly (%)[b]		
Yes	14 (37.8)	19 (46.3)
None	23 (62.2)	22 (53.7)
Work-year[a]	2.11 ± 1.09	3.17 ± 1.88*
Working department[b]		
Blue-collar workers (%)	21 (56.8)	35 (85.4)
White-collar workers (%)	16 (43.2)	6 (14.6)
Used respiratory equipment[b]		
Yes (%)	30 (81.1)	38 (95.0)
No (%)	7 (18.9)	2 (5.0)
Used gloves[b]		
Yes (%)	17 (45.9)	23 (57.5)
No (%)	20 (54.1)	17 (42.5)

[a]Data [means ± (standard deviation)] were analyzed using the Wilcoxon rank sum test; [b]Number (% of total subjects) were analyzed using Fisher's exact test; *: $p<0.05$.

Table 2. Difference of 8-OH-dG and MDA levels of workers between bottom and fly ash treatment plants

	Bottom ash (n=37)	Fly ash (n=41)	*p*-value
8-OH-dG (μg/g creatinine)[a]	7.34 ± 12.11 (0.04–49.79)[b]	4.06 ± 1.88 (1.13–9.14)[b]	0.079
MDA (μM)[a]	0.58 ± 0.36 (0.09–1.47)[b]	3.20 ± 1.22 (1.33–7.20)[b]	< 0.0001*

[a]mean ± SD (Wilcoxon rank sum test); [b]min-max; *$p<0.05$.

Results

Demographic distributions and occupational histories of workers

Workers' average working duration at the fly ash plant (3.17 yr) was significantly longer than that at the bottom ash plant (2.11 yr). However, the workers at the two plants did not differ significantly in age, gender ratio, smoking status, or distribution of their working departments. However, more workers used respiratory equipment (95.0%) in the fly ash treatment plants than those in the bottom ash recovery plant (81.1%) (Table 1).

Oxidative stress markers

We found significantly higher MDA levels in fly ash treatment plant workers than in bottom ash plant workers (3.20 μM versus 0.58 μM, respectively; $p<0.0001$). The difference in 8-OH-dG level between the two types of plant was marginally significant ($p=0.079$; Table 2).

Working status and oxidative stress markers

All workers were categorized, based on their job titles

Table 3. 8-OH-dG and MDA levels of different working department workers in bottom and fly ash treatment plants

	Blue-collar workers			White-collar workers		
	Bottom ash (n=21)	Fly ash (n=35)	*p*-value	Bottom ash (n=16)	Fly ash (n=6)	*p*-value
8-OH-dG[a]	7.30 ± 12.52	4.14 ± 1.88	0.148	7.38 ± 11.95	3.63 ± 2.04	0.555
MDA[b]	0.56 ± 0.37	3.24 ± 1.26	< 0.0001*	0.60 ± 0.35	2.98 ± 0.96	0.0004*

[a]μg/g creatinine, mean ± SD (Wilcoxon rank sum test); [b]μM, mean ± SD; (Wilcoxon rank sum test); *p<0.05.

Table 4. The influence of working years on MDA and 8-OH-dG between bottom and fly ash treatment plants

	Work years < 2.45[#]			Work years ≥ 2.45		
	Bottom ash (n=22)	Fly ash (n=17)	*p*-value	Bottom ash (n=15)	Fly ash (n=24)	*p*-value
8-OH-dG[a]	10.60 ± 14.78	4.29 ± 2.23	0.630	2.55 ± 2.83	3.90 ± 1.63	0.023*
MDA[b]	0.63 ± 0.40	3.04 ± 1.21	< 0.0001*	0.50 ± 0.29	3.32 ± 1.24	<0.0001*

[a]μg/g creatinine, mean ± SD (Wilcoxon test); [b]μM, mean ± SD (Wilcoxon test); *p<0.05. [#]: Median of work years in the workers of bottom ash and fly ash plants.

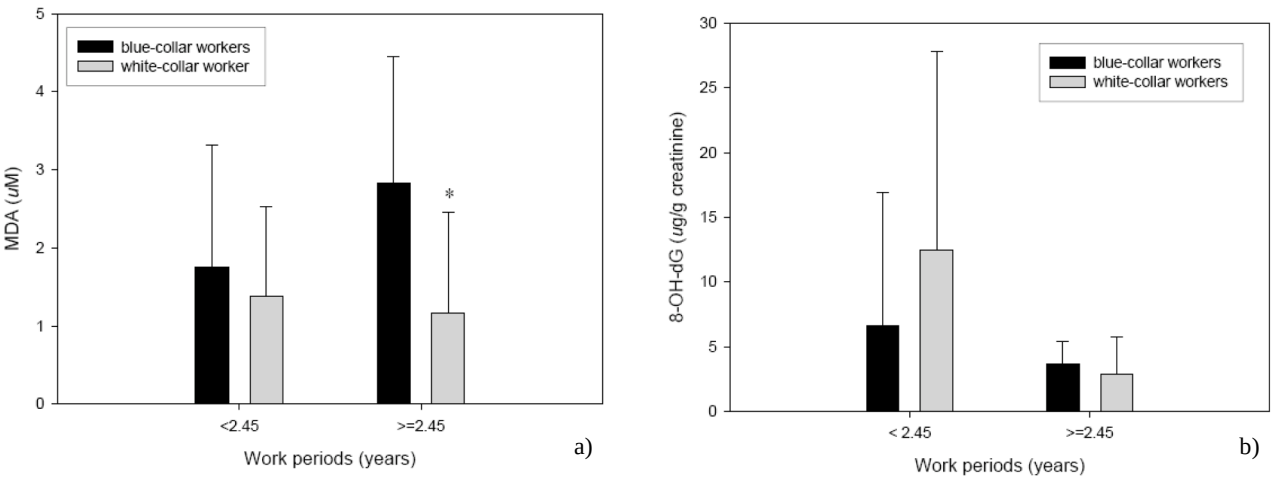

Fig. 1. a)MDA levels of blue-collar (factory-floor or ash) workers and white-collar (office) workers based on how many years they had been working at their factories. *p<0.05. b) 8-OH-dG levels of blue-collar workers and white-collar based on how many years they had been working at their factories.

or working departments, as members of blue-collar or white-collar groups in their respective plants. MDA levels were significantly higher in fly ash plant workers than bottom ash plant workers, regardless of whether they belonged to a blue-collar group or a white-collar group (Table 3). However, the levels of 8-OH-dG in this study were contradictory (p>0.05). In addition, MDA levels were significantly higher in fly ash plant workers than bottom ash plant workers, regardless of how long they had been working at the plant. Significantly higher levels of 8-OH-dG were found only in participants who had been working at the plants for more than 2.45 yr, the median work-years of the workers in the bottom ash and fly ash plants (Table 4). Significantly higher MDA levels were found in white-collar groups only among those who had been working longer than 2.45 yr (Fig. 1a). This finding was not consistent with 8-OH-dG levels in the workers (Fig. 1b).

Association between working status and oxidative stress markers

The data indicated a significant association between

Table 5. Multivariate regression of MDA levels and occupational status after adjusting for age, working period and smoking status

Variables			Regression	
Dependent	Independent	R^2	coefficient	*p*-value
MDA		0.735		
	Fly ash vs. bottom ash		0.805	< 0.0001*
	Office vs. production		–0.022	0.760
	Smoking		0.017	0.770
	Age		0.207	0.435
	Working period		–0.0003	0.998

*p<0.05.

the MDA levels of workers and their working plant, especially for fly ash treatment plant workers (Table 5).

Discussion

Environmental pollutants, such as TCDD, PAHs, and metals are abundant in waste incinerators[4, 6, 7] and contribute to the production of oxidative stress[16–21].

In the present study, we found that the levels of MDA in blood taken from fly ash treatment plant workers (3.20 μM) exceeded those taken from the bottom ash recovery plant workers (0.58 μM). We previously[22] found that the plasma MDA level of zinc recovery plant workers was 2.54 μM and that of copper smelting plant workers was 1.79 μM. The MDA levels in fly ash treatment plant workers were higher than those of workers at the zinc and the copper plants. Therefore, the data strongly indicate that fly ash treatment plant workers are exposed to chemicals that are much more hazardous than those in bottom ash, zinc recovery, and copper smelting plants. Although the fly ash plant workers had worked longer than the bottom ash plant workers, more bottom ash recovery plant workers than fly ash treatment plant workers were grouped as "blue-collar workers" (85.4% vs. 56.8%), but the interference of the working period and working styles on MDA levels disappeared after multivariate adjustment.

Lisk[23] reported that there might be much more arsenic, cadmium, nickel, lead, titanium, and zinc in fly ash and suspended particulates than in slag. Yoo *et al.*[24] also identified more volatile metals such as cadmium, lead, and zinc, which they found were more abundant in particulates emitted through stacks than in bottom ash. Kuo *et al.*[25] also reported that six metals (iron, aluminum, copper, zinc, chromium, and lead) were mostly concentrated in bottom ash, and that cadmium existed primarily in fly ash. The bottom ash from municipal solid waste incineration still contains a significant amount of small pieces of metals such as iron, copper, aluminum, lead, tin, zinc, and, and silver[26]. Large quantities of dioxins are leached from fly ash but not from bottom ash[27]. In addition, Chen *et al.*[22] found that scrubber ash was more toxic than bottom ash, and that bottom ash was more toxic than cyclone ash. According to the above studies, the contents of metals and dioxins in bottom ash and fly ash are different, and the data of the present study shows that oxidative damage differed between bottom and fly ash workers.

Yoshida *et al.*[14] found that the longer individuals worked in jobs that were exposed to fly ash from municipal solid waste incineration, the higher their urinary 8-OH-dG was. However, in the present study, we found that 8-OH-dG levels were higher in bottom ash recovery workers than in fly ash treatment workers, but only for those who had been occupationally exposed for more than 2.45 yr. We found a larger variation in urinary 8-OH-dG levels in bottom ash workers than in fly ash workers, and previous studies[15, 28] found that smoking status and alcohol consumption were also related to 8-OH-dG levels. Therefore, the association between occupational exposure and 8-OH-dG level may be affected by the life style of the workers.

Conclusion

The present study showed that fly ash treatment plant workers had significantly higher plasma MDA than bottom ash plant workers, and that a positive correlation existed between working at a fly ash plant and serum MDA levels. Together with previous findings that larger quantities of dioxins and metals are leached from fly ash than from bottom ash, the data indicate elevated oxidative stress in fly ash treatment plant workers in Taiwan.

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