Letter to the Editor

Dynamic Changes in DNA Damage and Repair Biomarkers with Employment Length among Nickel Smelting Workers*

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Our study explored the dynamic changes in and the relationship between the DNA damage marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the DNA repair marker 8-hydroxyguanine DNA glycosidase 1 (hOGG1) according to the length of occupational employment in nickel smelting workers. One hundred forty nickel-exposed smelting workers and 140 age-matched unexposed office workers were selected from the Jinchang cohort. The 8-OHdG levels in smelting workers was significantly higher than in office workers (*Z*=-8.688, *P*<0.05) and the 8-OHdG levels among nickel smelting workers in the 10-14 y employment length category was significantly higher than among all peers. The hOGG1 levels among smelting workers were significantly lower than those of non-exposed workers (*Z*=-8.948, *P*<0.05). There were significant differences between employment length and hOGG1 levels, with subjects employed in nickel smelting for 10-14 y showing the highest levels of hOGG1. Correlation analysis showed positive correlations between 8-OHdG and hOGG1 levels (*r*=0.413; *P*<0.01). DNA damage was increased with employment length among nickel smelting workers and was related to the inhibition of hOGG1 repair capacity.

Nickel and its compounds are important industrial poisons and environmental pollutants that can cause harm to human health and result in lung cancer, particularly among those who have engaged in smelting operations. One case-control study found that the risk of lung cancer was 20 times higher in electrolytic workers than in the general population[1]. Additionally, nickel-smelting workers were found to have a significantly high mortality rate from lung cancer in a retrospective cohort study based in Jinchang[2]. Currently, the molecular mechanisms by which nickel compounds cause lung cancer remain unclear, but research on the intermediate causal mechanisms between nickel exposure and lung cancer have shown that DNA damage by nickel compounds is an important carcinogenic mechanism[3].

Environmental nickel exposure can cause oxidative stress and oxidative damage in the body, inducing the production of reactive oxygen species (ROS)[4]. Excessive ROS accumulation can cause DNA oxidative damage, producing modified nucleotides including 8-hydroxy-2'-deoxyguanosine (8-OHdG). An elevated 8-OHdG level is regarded as an indicator of DNA oxidative damage. Xu et al. observed that nickel exposure can cause increases in 8-OHdG levels in mouse neuroblastoma cell lines, indicating DNA damage[4]. However, the temporal trend of 8-OHdG with length of employment and occupational exposure has not previously been studied in a nickel exposure cohort.

The product of ROS attack on genomic DNA is the premutagenic lesion 7,8-dihydro-8-oxoguanine (8-oxoG), which causes G-to-T transversions. 8-oxoG is primarily removed by the base excision repair (BER) pathway. Human 8-oxoguanine DNA glycosylase (hOGG1) is a single base excision repair enzyme, specifically recognizing and repairing DNA oxidative damage by removing 8-OHdG[5]. The oxidative stress generated by nickel exposure cause oxidative DNA damage, and heavy metals are considered inhibitors of oxidative DNA repair pathways[6]. However, nickel compounds

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have not been previously related to changes in hOGG1 levels in cohort studies.

Research on the effects of nickel exposure on DNA damage and repair capacity in occupational populations is currently lacking, especially with regard to the dynamic variation of DNA damage and repair indicators relative to the length of employment. The DNA oxidative damage marker 8-OHdG and the repair marker hOGG1 were measured in order to analyze the effect of exposure time on DNA oxidative damage and repair capacity in order to provide data on the molecular mechanisms of DNA damage and repair, to explain the high incidence of cancer in this cohort, and to identify early warning biomarkers.

The study population was based on the Jinchang cohort, which includes about 50,000 employees who underwent health examinations and epidemiological surveys from June 2011 to December 2013[2]. Nearly 5000 male workers at the Jinchuan Nonferrous Metals Corporation are engaged in the nickel smelting process. For blood samples, 140 nickel-exposed workers were selected from among the 969 smelting workers; based on their employment history, the workers were divided into seven employment length groups (0-4 y, 5-9 y, 10-14 y, 15-19 y, 20-24 y, 25-29 y, and 30-34 y), with 20 workers in each group. An age-matched control group of 140 nickel-unexposed office workers was formed and divided in the same way. In order to remove confounding by subject age in the relationship between the length of occupational exposure and DNA damage repair, the employment length groups were made to correspond to worker age. The levels of 8-OHdG and hOGG1 were measured with an enzyme-linked immunosorbent assay (ELISA) using commercial kits (ShangHai JingTian Biotechnology Co., Ltd.; R&B Co., Prod. No. 1R941).

Urine samples were collected from 100 workers among the same nickel smelting workers group, including 20 samples from each of five employment length groups (5-9 y, 10-14 y, 15-19 y, 20-24 y, and 25-29 y). Controlling for the effect of subject age, samples from 50 office workers including 10 from each employment length category were used for comparison. Urine samples were not available for the 0-4 y and 30-34 y employment length groups, resulting in a different number of blood and urine samples. Nickel in the urine samples of both smelting workers and office workers was detected using inductively coupled plasma mass spectrometry (Agilent 7700). This study was approved by the ethics committee of Lanzhou University.

Statistical analyses were performed by using the Mann-Whitney average rank test and variance (ANOVA); $P<0.05$ was considered significant. All data are expressed as mean±standard deviation (SD).

This study found that urine nickel concentrations are higher among smelting workers than office workers in each of the five employment length categories (Table 1), suggesting that the distribution of different species of nickel may affect the nature of exposure among nickel smelting workers. Previous studies have shown that considerable amounts of soluble nickel aerosols are likely to be present in occupational situations[7], and a strong correlation has been found between exposure to soluble nickel aerosols and urinary nickel concentrations. Urinary nickel concentration is usually used to assess nickel exposure levels[8]. We found that there was no relationship between urinary nickel concentrations in smelting workers and length of employment, indicating that nickel may not accumulate in the body and practically the entire absorbed amount is excreted in the urine.

This study shows that 8-OHdG levels in smelting workers were significantly higher than in office workers, and therefore DNA oxidative damage increases with length of employment and exposure in

Table 1. Urinary Nickel Concentrations between Nickel Smelting Workers and Office Workers at Different Lengths of Employment

Length of Employment (years)	Nickel Smelting Workers (μg/L)	Office Workers (μg/L)
5-9	9.246±4.690*	3.921±1.502
10-14	11.558±6.338*	1.924±1.259
15-19	14.300±13.970*	3.698±2.073
20-24	11.282±7.855*	3.228±2.162
25-29	11.613±4.963*	4.795±3.372
Total	11.690±8.281*	3.523±2.311
P (Mann-Whitney)	-8.277	
P	0.000**	
r	0.113	0.148
P	0.265	0.305

Note. *$P<0.05$ comparing levels in the nickel-exposed and unexposed workers in the specified employment length category; **$P<0.05$ comparing the levels in the nickel-exposed and unexposed workers.

nickel smelting workers (Table 2). These findings suggest that nickel compounds can indeed cause DNA damage due to chronic exposure in smelting workers. However, 8-OHdG levels were elevated to a degree in unexposed groups, indicating that normal cells may produce 8-OHdG in the metabolic processes related to cell apoptosis[9]. Our study also shows that 8-OHdG levels vary dynamically with exposure time among smelting workers, peaking in the third employment length category before decreasing. This suggests that the third employment length category (10-14 y) is the most serious period of oxidative DNA damage. Rather than noting a continued increase in 8-OHdG levels with employment beyond 15 y, suggesting an accumulation of DNA damage, the downward trend in 8-OHdG levels among nickel smelting workers approaching the levels experienced by office workers suggests that cellular mechanisms are gradually able to adapt to constant levels of nickel exposure, allowing the body to develop tolerance and resist the damaging effects of nickel exposure.

Table 2. Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) Concentrations in Nickel Smelting Workers and Office Workers at Different Lengths of Employment

Length of Employment (years)	Nickel Smelting Workers (μg/L)	Office Workers (μg/L)
0-4	0.179±0.023*	0.147±0.012
5-9	0.169±0.029*	0.144±0.012
10-14	0.184±0.023*	0.144±0.012
15-19	0.165±0.027*	0.141±0.012
20-24	0.163±0.021*	0.145±0.017
25-29	0.155±0.020	0.147±0.017
30-34	0.163±0.019*	0.147±0.018
Total	0.168±0.025	0.145±0.014
Z	-8.688	
P (Mann-Whitney)	0.000**	
F	3.524	0.600
P (ANOVA)	0.003**	0.730
r	-0.239	-0.026
P (Spearman)	0.005***	0.759

Note. *P<0.05 comparing levels in the nickel-exposed and unexposed workers in the specified employment length category; **P<0.05 comparing levels in the nickel-exposed and unexposed workers; ***P<0.05 comparing each group among different employment length categories.

Our results show that hOGG1 levels in smelting workers were significantly lower than in office workers, suggesting that nickel compounds mediated DNA oxidative damage by inhibiting DNA repair processes. Nickel has been found to inhibit DNA damage repair[6], thus indirectly allowing for the accumulation of DNA oxidative damage and thereby increasing the risk of cancer[10]. hOGG1 is inhibited by even relatively short durations of nickel exposure, as noted in the 0-4 y exposure group. After DNA damage reaches a certain threshold in the 10-14 y exposure group, as indicated by elevated 8-OHdG levels, hOGG1 expression is increased in nickel smelting workers, but is still lower than that seen in office workers. Correlation analysis found that 8-OHdG levels were positively related to hOGG1 levels (r=0.413, P<0.01), with synchronized dynamic changes following different lengths of employment. The levels of 8-OHdG and hOGG1 were found to change with the length of exposure time among nickel smelting workers in this study, peaking in the third employment length category, suggesting that induction of oxidative stress by nickel compounds resulting in DNA damage is most serious after 10-14 y.

Table 3. Serum 8-hydroxy-guanine DNA Glycosidase 1 (hOGG1) Concentrations in Nickel Smelting Workers and Office Workers at Different Lengths of Employment

Length of Employment (years)	Nickel Smelting Workers (U/L)	Office Workers (U/L)
0-4	0.057±0.045*	0.091±0.047
5-9	0.059±0.011*	0.096±0.069
10-14	0.077±0.061	0.097±0.072
15-19	0.058±0.009*	0.102±0.077
20-24	0.048±0.020*	0.081±0.021
25-29	0.052±0.005*	0.082±0.029
30-34	0.046±0.010*	0.094±0.027
Total	0.057±0.031	0.092±0.053
Z	-8.948	
P (Mann-Whitney)	0.000**	
F	2.194	0.425
P (ANOVA)	0.047***	0.861
r	-0.064	0.137
P (Spearman)	0.046***	0.107

Note. *P<0.05 comparing levels in the nickel-exposed and unexposed workers in the specified employment length category; **P<0.05 comparing levels in the nickel-exposed and unexposed workers; ***P<0.05 comparing levels in each group among different employment length categories.

Simultaneously, the repair process was initiated in response to oxidative damage, leading to the highest levels of hOGG1 among smelting workers, but at levels still significantly lower than those seen in unexposed office workers due to inhibition of the body's repair process by nickel compounds.

This study is the first report of synchronous dynamic trends in the DNA damage biomarker 8-OHdG and the DNA repair biomarker hOGG1 in nickel smelting workers. DNA damage increased with length of employment, peaking at 10-14 y of exposure. Ten to fourteen years of chronic exposure may represent a critical point of DNA damage in nickel smelting workers. The study also found that DNA damage in nickel smelting workers was related to the inhibition of DNA damage repair capacity.

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