

RESEARCH ARTICLE

Histone Methylation in Nickel-Smelting Industrial Workers

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Abstract

Background

Nickel is an essential trace metal naturally found in the environment. It is also common in occupational settings, where it associates with various levels of both occupational and non-occupational exposure. *In vitro* studies have shown that nickel exposure can lead to intracellular accumulation of Ni²⁺, which has been associated with global decreases in DNA methylation, increases in chromatin condensation, reductions in H3K9me2, and elevated levels of H3K4me3. Histone modifications play an important role in modulating chromatin structure and gene expression. For example, tri-methylation of histone H3k4 has been found to be associated with transcriptional activation, and tri-methylation of H3k27 has been found to be associated with transcriptional repression. Aberrant histone modifications have been found to be associated with various human diseases, including cancer. The purpose of this work was to identify biomarkers for populations with occupational nickel exposure and to examine the relationship between histone methylation and nickel exposure. This may provide a scientific indicator of early health impairment and facilitate exploration of the molecular mechanism underlying cancer pathogenesis.

Methods

One hundred and forty subjects with occupational exposure to Ni and 140 referents were recruited. H3K4 and H3K27 trimethylation levels were measured in subjects' blood cells.

Results

H3K4me3 levels were found to be higher in nickel smelting workers (47.24±20.85) than in office workers (22.65±8.81; $P = 0.000$), while the opposite was found for levels of H3K27me3 (nickel smelting workers, 13.88± 4.23; office workers, 20.67± 5.96; $P = 0.000$). H3K4me3 was positively ($r = 0.267$, $P = 0.001$) and H3K27 was negatively ($r = -0.684$, $P = 0.000$) associated with age and length of service in smelting workers.

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Conclusion

This study indicated that occupational exposure to Ni is associated with alterations in levels of histone modification.

Introduction

Nickel is one of the most abundant trace metals on Earth, and is widely distributed in the general environment. It is used in various occupational settings, including in mining, smelting, and refining and in the alloy production and welding industries[1]. Workers employed in these industries are chronically exposed to nickel and related compounds, and non-occupational exposure is also common. Exposure may occur via inhalation of nickel-contaminated dust or air, ingestion of contaminated food or water, or other environmental sources such as tobacco and consumer products like stainless steel kitchen utensils and jewelry[2]. Existing epidemiological and experimental data have suggested that nickel, and other substances in the exposed environment can increase the risk of lung cancer and that nickel and various nickel compounds are carcinogens in humans[3,4]. Lung cancer is the leading cancer in China. Recent epidemiological investigations have revealed that occupational exposure to nickel is significantly closely associated with increased mortality of lung cancer among nickel smelting industry workers in China[5]. Over the past three decades, the morbidity and mortality associated with lung cancer have increased, bringing the overall 5-year survival rate down to below 15%, so better methods of early detection and treatment are needed[6].

Given that nickel and its compounds have been shown to have weak mutagenic capacity in bacterial and mammalian cell culture systems, the role of epigenetics has drawn attention as a direction to be explored[7]. Histone modifications play an important role in many nuclear processes, such as modulation of chromatin structure and gene transcription[8]. Tri-methylation of the histone H3k4 has been found to be associated with transcriptional activation, and tri-methylation of H3k27 has been found to be associated with transcriptional repression [9]. Aberrant histone modifications have been associated with various human diseases, including cancer[10]. *In vitro* studies have shown that nickel exposure can lead to intracellular accumulation of Ni²⁺, which is associated with global decreased DNA methylation, increased chromatin condensation, reduced H3K9me2, and elevated H3K4me3[11,12]. Most previous studies of histone modifications in subjects exposed to nickel have been case-control studies and animal experiments. Although there have been numerous cohort studies on nickel exposure, few have focused on changes in histone modifications. For this reason, the present cohort study focused on nickel-associated changes in global histone modifications using peripheral blood cells from nickel smelting factory workers in Jinchang, China. The purpose of this study was to identify biomarkers in populations with occupational nickel exposure, to determine the relationship between length of industrial service and nickel exposure, to provide a scientific indicator of early health impairment, and to explore the possible molecular mechanisms underlying the pathogenesis of cancer.

Methods

Ethics statement

Protocols involving this human cohort study were approved by the Ethics Committee of Lanzhou University. All participants provided written informed consent to their participation in this study.

Table 1. Sampling distribution of high nickel exposure in groups with different lengths of service.

Years of Service	Age group (years)						
	20–24	25–29	30–34	35–39	40–44	45–49	50 and older
0–	20	—	—	—	—	—	—
5–	—	20	—	—	—	—	—
10–	—	—	20	—	—	—	—
15–	—	—	—	20	—	—	—
20–	—	—	—	—	20	—	—
25–	—	—	—	—	—	20	—
30+	—	—	—	—	—	—	20

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Subjects

Healthy subjects who had undergone a physical examination at the employee health center of Jinchuan Corporation or its secondary branches from June 1, 2011 to January 31, 2012 were enrolled in the present study (N = 12,388 subjects; 8,434 male).

To identify differences among nickel-smelting workers, subjects from secondary branches with the highest nickel exposure (nickel smelting factories), were included in the exposed group. Office workers were included in the control group.

Exposed worker sampling

High-exposure positions in the company were identified using technical personnel familiar with the production process and the type and concentration of nickel compounds in the working environment. Among the 3,649 smelting workers, 969 had occupational exposure to high doses of nickel. The distribution of workers based on length of service (5-year increments) and age groups are shown in [Table 1](#). To eliminate the confounding effects of age from the relationship between length of service and nickel exposure, a diagonal sampling method was used to select 20 subjects in each group for sampling (total subjects in exposed group = 140).

Control sampling

Office workers age-matched to subjects in the different exposure subgroups (within 3 years of difference) were selected. Seven age groups (5-year increments) with 20 subjects each were used (N = 140 control subjects).

Division of job, occupational history, and calculation of service length

All workers enrolled in the initial survey completed a detailed occupational history with a chronological summary of work in any secondary branches of the Jinchuan Corp. All subjects selected for the exposure group had been employed in smelting factories as a first job and the length of service was defined as years worked at positions with exposure to high concentrations of nickel.

Blood sample collection and handling

Fasting blood samples were collected in the morning and split into four aliquots with two anti-coagulant and two nonanticoagulant collections (3 ml/each collection) for each recruited subject. After gently shaking, blood samples were centrifuged by a low-temperature ultracentrifuge at 2000 mp/min for 10 min at 4°C. Then 1 ml of serum or plasma was transferred to 1.8 ml

frozen tubes and stored at -80°C . Blood samples were stored at 4°C and left at room temperature for 1 h before histone extraction using histone extraction kit (manufactured by Epigentek Group Inc. and the Cat. Log NO is OP-0006).

Histone extraction

Histone extraction was performed according to the manufacturer's instructions. Briefly, the histone extraction kit was left at room temperature to balance the temperature between the reagents and blood samples. Then 300 μl of serum or plasma was lysed with equal volume of cell lysis buffer from the kit. The lysate was gently mix for 10 min on ice followed by 10,000 rpm/min centrifugation at 4°C for 1 minute. The pellet was resuspended with 300 μl lysis buffer and incubated on ice for 30 min. This was followed by 12,000 rpm/min centrifugation at 4°C for 5 min. The supernatant was transferred into a new Eppendorf tube and diluted DTT buffer was added at a ratio of 0.3: 1 (0.3 ml diluted DTT in 1 ml supernatant). Samples were vortexed followed by 95°C incubation for 40 min. Samples were then cooled down with flowing water followed by 4000 rpm/min centrifuge for 10 min. The supernatant was measured for OD value at 532 nm with ddH₂O as a blank control.

Detection of global histone modification

Histone modifications were assessed using a sandwich enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

After natural logarithm conversion, the H3K27me₃ and H3K4 me₃ were found to fit normal distribution, So the data are here expressed as mean \pm standard deviation ($\bar{x} \pm s$). Differences in histone modifications in blood cells among groups were compared using two-sample Student's t-test, Differences in histone modifications in blood cells between people with different lengths of service were compared using the F test.

Results

General information of the subjects from the nickel-smelting industry

In the experimental exposure group (nickel-smelting workers), 140 male subjects ranging in age from 20–55 years old (38.34 ± 8.92) were recruited. In the control group (office workers), 140 male subjects ranging in age from 24–55 years old (38.34 ± 8.89) were recruited. All subjects were healthy with no chronic diseases and no recent history of any medication.

Analysis of the effects of time on tri-methylation of histone in the high-nickel-exposure group

After natural logarithm conversion, the H3K4me₃ data were found to fit normal distribution, there was heterogeneity of variance. For all length of service subgroups, t' test indicated that there were significant differences in H3k4me₃ level between the nickel-smelting workers and office workers ($P < 0.05$) (Table 2). The level of H3k4me₃ in subjects with high occupational exposure to nickel was significantly higher than in the control group ($P < 0.05$) (Figs 1 and 2). In the exposed group, the H3k4me₃ levels in the 30+ service length subgroup were the highest, reaching a mean level of 58 nmol/mg prot. In the other service groups, H3k4me₃ levels increased gradually with the length of service, and there was a significant positive correlation between the level of H3k4me₃ and the length of service ($r_s = 0.267$, $P = 0.001$). In the control

Table 2. H3K4me3 in nickel smelting and office workers with different lengths of service (nmol/mg prot).

Groups	working years (20 individuals per group)							Total	r_s	P
	0–4.99	5–9.99	10–14.99	15–19.99	20–24.99	25–19.99	30+			
Smelting	37.71±1.13	44.26±1.22	36.23±1.11	45.15±1.58	49.90±1.09	42.52±2.01	52.98±1.52	47.24±20.85	0.267	0.001*
Office	13.60±1.54	16.28±1.31	20.09±1.60	20.70±1.51	23.34±1.36	25.79±1.28	31.19±1.11	22.65±8.81	0.608	0.000*
t'	10.320	11.372	6.514	5.015	12.995	3.167	4.427	12.859		
P	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*		

Note: * $P < 0.05$ was here considered statistically significant.

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group, the level of H3k4me3 was also positively correlated with the length of service ($r_s = 0.608$, $P = 0.000$).

As illustrated in Fig 2, in the first service length subgroup (< 5 years), the level of H3K27me3 of nickel-smelting workers was comparable to that of office workers (Fig 3). Starting from the

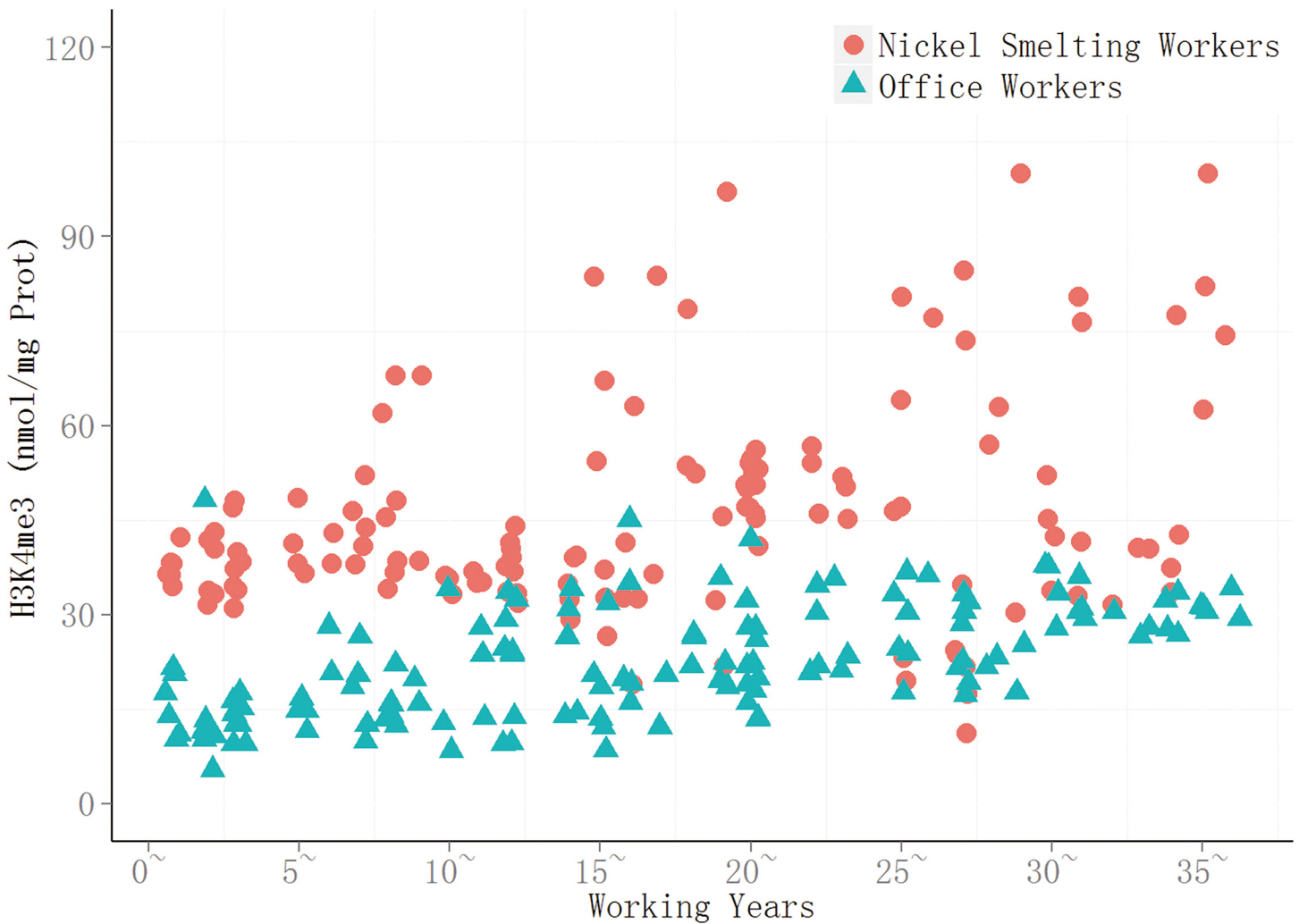


Fig 1. H3K4me3 in smelting and office workers with different working years. The level of H3K4me3 in every experimental subject, the red balls express the smelting workers, and green triangles express the office workers.

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10–14.99 service length subgroup, the levels of H3K27me3 of nickel-smelting workers were notably lower in the scatter plot and were distinctly different from those of office workers (Fig 3).

After natural logarithm conversion, the H3K27me3 data were found to fit normal distribution. A homogeneity of variance test for different length of service subgroups in the high exposure group showed $F = 1.121$ and $P = 0.353 > 0.05$, and a test of the control group showed $F = 1.234$ and $P = 0.293 > 0.05$. In this way, in both cases, there was homogeneity of variance.

As shown in Table 3, In each length of service subgroup, the level of H3K27me3 of nickel-smelting workers was significantly different from that of office workers from the corresponding subgroup ($P < 0.05$), and the level of H3K27me3 of office workers was higher than that of nickel-smelting workers.

Discussion

Previous epidemiological investigations on human subjects and experimental results from cell lines and animal models have indicated that nickel and nickel-containing compounds are

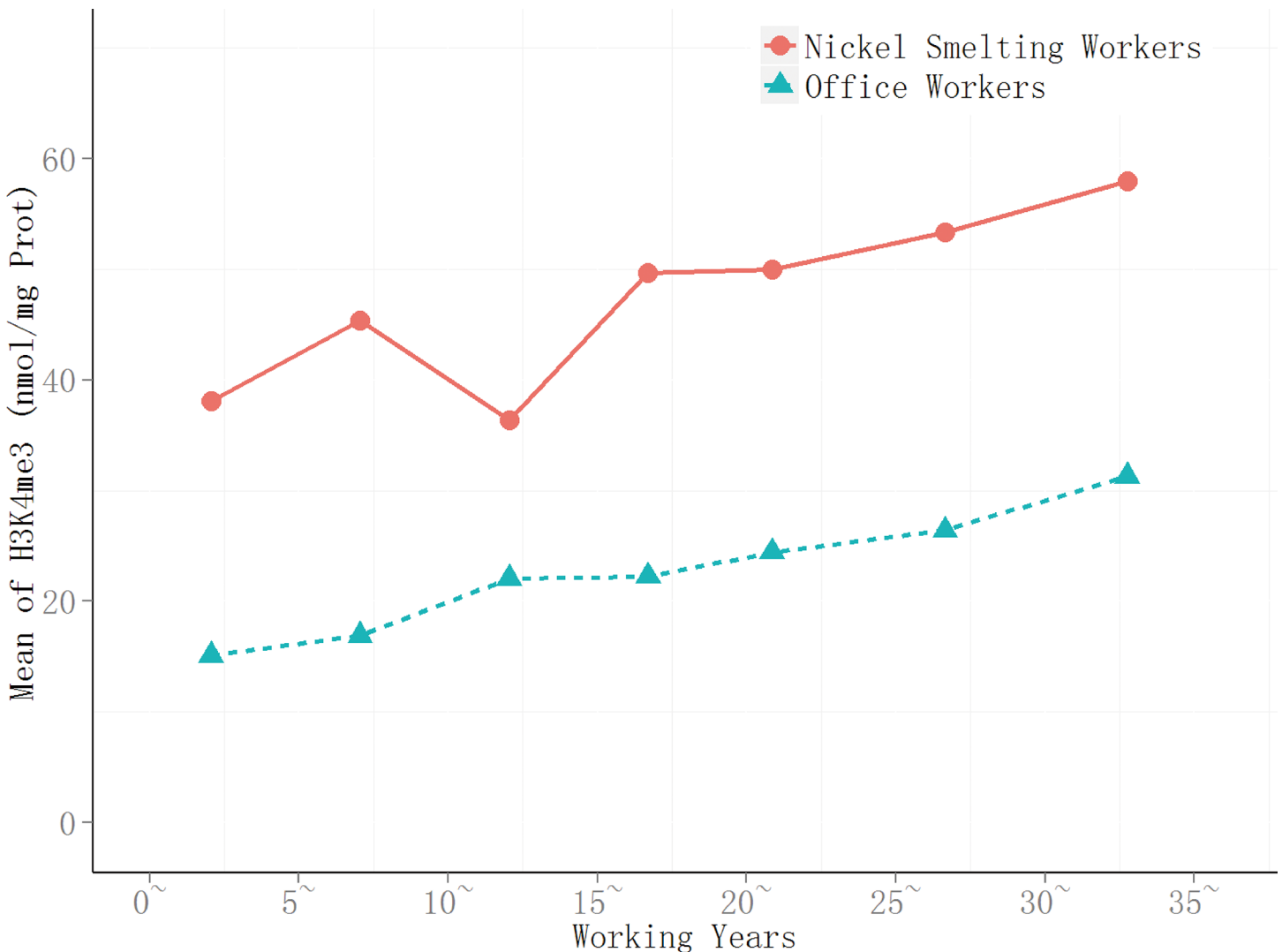


Fig 2. Trend of H3K4me3 with increasing working years. The trend of H3K4me3 in two group with increasing working years, the line connected with red balls express the smelting workers, and with green triangles express the office workers.

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Fig 3. H3K27me3 in smelting and office workers in different working years. The level of H3k27me3 in every experimental subject, the red balls express the smelting workers, and green triangles express the office workers.

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mutagens and carcinogens[13–15]. However, the exact molecular mechanism underlying nickel carcinogenicity remains unclear.

Given the low mutagenic capacity of nickel and its compounds, epigenetic changes may offer an alternative explanation for nickel-associated cancer development and provide pre-

Table 3. H3K27me3 in nickel smelting and office workers with different lengths of service (nmol/mg prot).

Groups	working years (20 persons in each group)							Total	r _s	P
	0–4.99	5–9.99	10–14.99	15–19.99	20–24.99	25–19.99	30+			
Smelting	19.56±1.24	16.58±1.26	13.09±1.18	13.04±1.31	11.99±1.22	10.67±1.33	9.96±1.24	13.88±4.43	-0.684	0.000*
Office	24.77±1.00	26.90±1.13	21.45±1.27	20.78±1.20	18.63±1.28	15.17±1.22	14.20±1.24	20.67±85.96	-0.709	0.000*
t	12.144	67.485	57.568	41.834	38.437	20.483	26.879	-10.813		
P	0.001*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*		

Note: *P < 0.05, statistically significant.

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clinical biomarkers[16]. In the present cohort study, global changes in histone modification were investigated in response to nickel exposure using peripheral blood cells from nickel-smelting and office workers in nickel-smelting factory. Length of service was found to be associated with increased levels of H3K4me3 (Figs 1 and 2) in both nickel-smelting and office workers, this may be taken as an effect of different levels of exposure to nickel, or partly an effect of age. The effect of nickel exposure was supported by recent cohort study using peripheral blood mononuclear cells from nickel refinery workers[9]. The global level of H3K27me3 was found to be significantly decreased in response to nickel exposure overtime (Figs 3 and 4). The level of H3K4me3 was found to be positively associated with the duration of nickel exposure (Figs 1 and 2), but the level of H3K27me3 was negatively associated with the duration of nickel exposure (Figs 3 and 4) for both nickel-smelting and office workers. Results showed that the levels of H3K4me3 and H3K27me3 were significantly different between nickel-smelting and office workers exposed for the same length of time (Figs 1 and 2), suggesting a dosage effect on those histone modifications. These results suggest that the global level of H3K4me3 and H3K27me3 would provide one of potential biomarkers for nickel exposure in a manner dependent on both length of exposure and dosage.

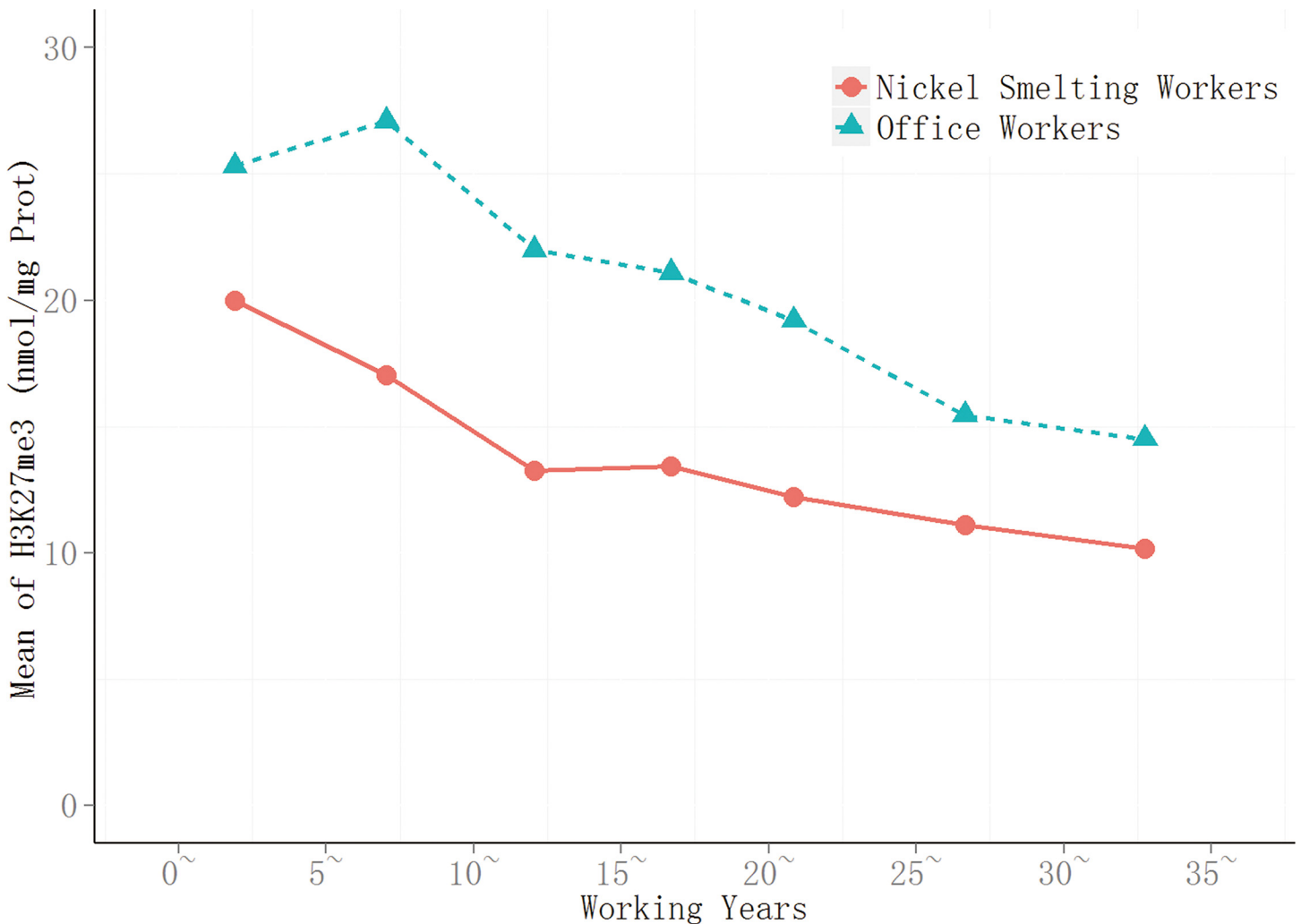


Fig 4. Trend of H3K27me3 with increasing working years. The trend of H3K27me3 in two group with increasing working years, the line connected with red balls express the smelting workers, and with green triangles express the office workers.

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Epigenetics has been shown to play a critical role in cancer development by changing the state of chromatin, activating oncogenes, and inhibiting the expression of tumor suppressor genes[17]. H3K4me3 has been shown to be associated with gene activation, while H3K27me3 has been shown to be associated with gene repression[18,19]. Deregulation of both histone methylation and the mutation of their regulatory enzymes, such as Mll family members for H3K4 methylation and polycomb repressive complexes (PRC) for H3K27 methylation, has been observed in the development of various cancers[20,21]. Probably due to the weak mutagenicity of nickel and its compounds epigenetic changes have received increasing scrutiny in nickel carcinogenesis studies, but little emphasis was placed on histone acetylation, methylation ubiquitination, DNA methylation, or the regulation of transcription factors[22–24]. Nickel exposure has been shown to be associated with decreased histone acetylation and increased H3K4me3 mark globally[9]. Functionally, both the phenotype and gene expression profile of nickel-transformed cells were reversed to that of untransformed cells by treatment with HDAC inhibitor trichostatin A (TSA), suggesting that nickel exposure might lead to the inhibition of histone acetylation during transformation[25]. Mechanistically, Ni²⁺ has been shown to compete with iron *and with* a co-factor of Jumonji-domain-containing histone demethylase (JMJD) family members *in vitro*. This inhibits KDM3A/JMJD1A activity and results in the increased H3K9 methylation upon exposure to nickel or its compounds[26]. It is here speculated that nickel exposure has similar effect on H3K4 demethylase, such as KDM5A/JARID1A, to repress its activity towards H3K4me3 and lead to the increased H3K4me3. In addition, nickel is a hypoxia mimetic and H3K4me3 has been shown to be increased in hypoxia environment through inhibiting the activity of H3K4 demethylase KDM5A/JARID1A[27]. Consistent with experimental results and mechanisms derived through *in vitro* assays, the first cohort study recently showed the increased H3K4me3 in human blood cells from nickel finery workers. This was here replicated using human blood cells from subjects in the nickel-smelting industry. New biomarkers for global decreased H3K27me3 should be identified. Furthermore, current results suggested the change of global level of H3K4me3 and H3K27me3 to be possibly associated with both length and dosage of nickel exposure. In line with these findings, exposure to Cr⁶⁺, a mutagen and carcinogen similar to Ni²⁺, has been shown to be associated with increased incidence of lung cancer[28], and with the elevation of H3K4me3 and reduction of H3K27me3 in human A549 cell line[29], suggesting similar histone modification changes in response to metal exposure may promote cancer development.

In summary, the current results showed the global increased H3K4me3 and decreased H3K27me3 to be associated with nickel exposure. Given that nickel exposure is associated with increased lung cancer, it is here speculated that aberrant H3K4me3 and H3K27me3 may change chromatin state more similar to that of cancer cells and therefore facilitate cancer development. Current findings suggest the change of histone methylation may provide one of potential biomarkers for early prediction and evaluation of nickel exposure-associated disease development. Further studies are expected to provide more insight into the mechanisms by which nickel exposure leads to lung cancer development for cancer prevention, diagnostics, and therapeutics. We had limited data on the different levels of nickel exposure from either measuring the levels of nickel in working places or the urinary nickel levels from subjects. Because of the lack of detailed information on nickel exposure, the study cannot establish a correlation between nickel air levels or urinary nickel levels and the changes in levels of histone modifications. Further, we have started collecting the first follow-up data for the “Jinchang cohort”[30], the urine samples have been collected and we are currently processing the samples for urinary nickel testing. Finally we have been trying our best to obtain the data regarding the levels of nickel in the air of various plants.

Supporting Information

S1 Dataset. The dataset is the OD of H3K27me3 and H3K4me3 by ELISA. And the group 1 express the smelting workers, the group 2 express the officers workers. (XLSX)

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Author Contributions

Conceived and designed the experiments: YB NC. Performed the experiments: LM. Wrote the paper: LM. Approved the manuscript for publication: LM YB NC HP FG MD HW JH TZ.

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