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ORIGINAL ARTICLE

Global DNA (LINE-1) methylation is associated with lead exposure and certain job tasks performed by electronic waste workers

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Abstract

Objective This study assessed the associations between blood and urine levels of toxic metals; cadmium (Cd) and lead (Pb), and methylation levels of the LINE-1 gene among e-waste and control populations in Ghana.

Methods The study enrolled 100 male e-waste workers and 51 all-male non-e-waste workers or controls. The concentrations of Cd and Pb were measured in blood and urine using inductively coupled plasma mass spectrometry, while LINE1 methylation levels were assessed by pyrosequencing of bisulfite-converted DNA extracted from whole blood. Single and multiple metals linear regression models were used to determine the associations between metals and LINE1 DNA methylation.

Results Blood lead (BPb) and urine lead (UPb) showed higher median concentrations among the e-waste workers than the controls (76.82 µg/L vs 40.25 µg/L, $p \leq 0.001$; and 6.89 µg/L vs 3.43 µg/L, $p \leq 0.001$, respectively), whereas blood cadmium (BCd) concentration was lower in the e-waste workers compared to the controls (0.59 µg/L vs 0.81 µg/L, respectively, $p = 0.003$). There was no significant difference in LINE1 methylation between the e-waste and controls ($85.16 \pm 1.32\%$ vs $85.17 \pm 1.11\%$, $p = 0.950$). In our single metal linear regression models, BPb was significantly inversely associated with LINE1 methylation in the control group ($\beta_{BPb} = -0.027$, 95% CI -0.045, -0.010, $p = 0.003$). In addition, a weak association between BPb and LINE1 was observed in the multiple metals analysis in the e-waste worker group ($\beta_{BPb} = -0.005$, 95% CI -0.011, 0.000, $p = 0.058$).

Conclusion Continuous Pb exposure may interfere with LINE1 methylation, leading to epigenetic alterations, thus serving as an early epigenetic marker for future adverse health outcomes.

Keywords Electronic waste · Toxic metals · DNA methylation · LINE-1

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Abbreviations

Cd	Cadmium
CpG	Cytosine-guanine dinucleotide
DNMTs	DNA methyltransferases
E-waste	Electronic waste
GeoHealth	Global environmental and occupational health
HEI	Health Effect Institute
LINE1	Long interspersed nucleotide element-1
NHANES	National health and nutrition examination survey
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
POPs	Persistent organic pollutants
SAM	S-adenosyl methionine

Introduction

Public health concerns due to the high production volumes of electrical and electronic waste (e-waste), especially in low- and middle-income countries, are well documented (Alabi et al. 2012; Baldé et al. 2017; Orlins and Guan 2016; Robinson 2009; Song and Li 2014). The composition of electrical and electronic equipment presents a challenge in the management of e-waste because it is simultaneously a source of recoverable precious materials (especially metals) as well as a myriad of toxic chemicals (Alabi and Bakare 2017; Amankwaa et al. 2017; Bakhiyi et al. 2018; Dias et al. 2019; Fowler 2017). Therefore, effective and adequate recycling processes are required to recover valuable materials while protecting human and environmental health (Ikhlayel 2017). This presents serious challenges for informal sector e-waste recycling facilities, especially in developing countries without appropriate recycling infrastructure (Ikhlayel 2018).

The high influx of second-hand electrical and electronic products into Ghana in recent years has resulted in a significant increase in the recycling and dumping of e-waste, which has offered employment opportunities to hundreds of young men in the capital Accra (Amankwaa et al. 2016). The Agbogbloshie e-waste processing and recycling site (a.k.a., scrapyard) is the main centre in Ghana for the recovery of reusable materials from e-waste. Agbogbloshie e-waste recyclers are a particularly vulnerable group because they are among the largest and busiest informal recyclers worldwide (Srigboh et al. 2016). The workers, often young men, are involved in multiple tasks and work in the open using rudimentary tools with little or no personal protective equipment. The recycling process itself involves the manual dismantling of old or end-of-use electronic and electrical equipment to retrieve reusable components. A significant activity at the e-waste recycling site involves open-air burning of electrical cables of all sizes in pits to retrieve oxidized copper wires with flammable materials such as plastics and foam recovered from old discarded fridges. This particular activity results in releasing a mixture of toxic chemicals such as PAHs, PCBs and toxic metals into the ambient environment. Several studies have documented high concentrations of PAHs, chlorinated and brominated dioxin-related compounds (DRCs) and dioxin-like polychlorinated biphenyls (DLPCBs), polybrominated diphenyl ethers (PBDEs) and toxic metals in surface soil samples from the Agbogbloshie e-waste recycling site in Ghana (Akortia et al. 2017; Daso et al. 2016; Tue et al. 2016, 2017). Other studies have found high levels of PAH-derived metabolites (Feldt, 2014) and toxic metals (Srigboh et al. 2016; Wittsiepe et al. 2017) in worker blood and urine.

Toxic metals are implicated in numerous adverse health outcomes, including cancers, cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction and autoimmune diseases (Hu 2002; Rzymski et al. 2015; Shi et al. 2019). Bal and colleagues extensively studied the genotoxicity aspect of metals. They reported that toxic metals largely influence their toxicity either through direct interaction with nuclear DNA or indirectly through generated reactive intermediates reacting with other cellular pathways such as inhibition of DNA repair mechanisms or both (Bal et al. 2011). Recent developments in the field of metal toxicity and carcinogenicity suggest that genetics alone cannot fully explain metal-induced chronic diseases, especially cancer, since most of the metals are weak mutagens. Epigenetic mechanisms such as DNA methylation; the most widely studied epigenetic marker, may in part mediate the health effects of occupational/environmental exposure to metals (Arita and Costa 2009).

Long interspersed nucleotide elements (LINE1) are repetitive elements or transposons that constitute approximately 18% of the human genome and are usually heavily methylated to ensure genomic stability and integrity (Kahl et al. 2019). The alteration of LINE1 is used as a proxy for global methylation changes (Ghosh et al. 2017; Sharma et al. 2019). Decreased levels of LINE1 methylation may result in increased mitotic recombination and overall genome instability (Kahl et al. 2019; Li et al. 2018), and this has been regarded as a biomarker of effect to different classes of xenobiotics in occupational settings (Kahl et al. 2019). A study among urban pesticide sprayers in Mexico showed decreased levels of LINE1 methylation among the pesticide-exposed group (Benitez-Trinidad et al. 2018). In another study in China where workers in a battery plant were occupationally exposed to Pb, LINE1 was inversely associated with Pb levels (Li et al. 2013). Duan and coworkers also reported hypomethylation of LINE1 among coke-oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) (Duan 2013).

Significan decrese in LINE1 methylation is observed in several types of cancers (Ehrlich 2002; Hsiung, 2007; Woo and Kim 2012; Zhu et al. 2011), and is regarded as a hallmark of cancer development (Buj et al. 2016; Das and Singal 2004). In a meta-analysis by Barchitta et al. (2014), the LINE-1 methylation level was significantly lower in cancer patients than in control samples ($p < 0.001$). The difference, however, was confined to tissue samples ($p < 0.001$) and not blood ($p = 0.23$).

Global DNA methylation changes associated with toxic metal exposure have been less clear and consistent, as demonstrated by studies showing both hypermethylation and hypomethylation. These studies were limited by differences in tissues examined, populations studied, methods for measuring methylation status, and singular analysis of the effect of one metal at a time (Bandyopadhyay et al. 2016; Goodrich et al. 2013; Hanna et al. 2012; Hossain et al. 2017, 2012; Lambrou et al. 2012; Li et al. 2013; Majumdar et al. 2010; Phetliap et al. 2018; Pilsner et al. 2009; Tajuddin et al. 2013; Tellez-Plaza et al. 2014; Wright et al. 2010). In addition, the informal e-waste recycling industry is largely understudied despite evidence that activities result in release and exposure to several toxic metals (Awasthi et al. 2016). There is little published data on the association between human metal exposure and DNA damage in the e-waste recycling industry (Alabi et al. 2019; Wang et al. 2011, 2018). To the best of our knowledge, no previous study has investigated the association between metal exposure to the e-waste workers themselves and DNA methylation changes; rather, these studies were carried out among non-worker populations residing near informal sector e-waste recycling (Li et al. 2020).

The objectives of this study were to (1) quantify the concentration of toxic metals in blood and urine (Cd and Pb), (2) evaluate LINE1 DNA methylation, and (3) assess the association between the concentration of blood and urine toxic metals and LINE1 DNA methylation among e-waste workers and a control group in Ghana. These metals were considered because of their widespread use in electrical and electronic products, their toxicity and public health significance (Tchounwou et al. 2012), and co-exposure risk (Fan et al. 2014).

Methods

Study area and population

The study was conducted in two locations: the e-waste recycling site in Agbogbloshie, Accra, Ghana, and Madina Zongo, a part of greater Accra located approximately 10 km from the e-waste site. The Agbogbloshie e-waste site is the largest and busiest e-waste dump in Africa (Srigboh et al. 2016). The site is situated on the banks of Korle Lagoon on the western side of the Odaw River in central Accra. To the east are various businesses, including banks, pharmaceutical companies, breweries, shops, and various manufacturing companies. To the south is a densely populated, 'resource-poor' community with most residents lacking access to essential services such as clean water and sanitation (Amankwaa et al. 2016). Agbogbloshie scrapyard is the main centre for the recovery of valuable materials from e-waste, with an estimated population of 80,000 (United Nations Population Fund 2018). The residents at the site are predominantly migrants from the northern parts of Ghana (Wittsiepe et al. 2017). The recycling process involves manual dismantling, collection, and sorting of electrical equipment and burning electrical and electronic items, including plastic materials such as wire insulation, which emit considerable smoke and pollute the local environment. The recyclers are mostly young men who use rudimentary tools such as a chisel, hammer, pliers, etc. or sometimes their bare hands with little or no protective equipment (Acquah et al. 2019).

The controls are residents at Madina Zongo in Accra, which is approximately 10 km north of Agbogbloshie. There are no e-waste recycling activities in the area, and individuals recruited are not involved in any e-waste work. Madina Zongo residents are known to be quite similar to e-waste workers with respect to the length of time residing in Accra and the region of the country from where they moved, socioeconomic background, religion, and culture.

Study design and participant recruitment

This study utilized existing data and specimens from the parent study (GeoHealth-II), a longitudinal repeated measures study with four rounds of data collection from Agbogbloshie workers and the comparison group in Madina Zongo. After completing a community entry process that included durbar that brought together researchers, leaders of the study sites, and e-waste workers, participants were recruited into the study. Each potential participant was given a detailed explanation of the study procedures and objectives, the benefits and possible risks of participating in the study, and was asked to provide written consent if willing to be enrolled. The inclusion criteria were adult males aged 18 years and above who have worked at the e-waste site for at least six months. The study was conducted after receiving ethical clearance from the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana (protocol identification number CHS-ET/M.4-P 3.9/2015–2016). Briefly, 151 participants were recruited from the first round of data collection from Agbogbloshie (100) and Madina Zongo (51). Due to higher than expected loss to follow-up rates in the second round of data collection, 56 new participants (Agbogbloshie = 42 and Madina Zongo = 14) were

recruited to address this challenge. Some participants provided multiple blood and urine samples during the four sampling periods from March 2017 to August 2018, bringing the total number of samples to 598. This study utilized data from the first sampling event (Agbogbloshie = 100 and Madina Zongo = 51) to investigate associations between heavy metal (Cd, Pb and As) and global (LINE1) methylation levels at baseline.

Data collection

Questionnaire survey

All participants answered a questionnaire that was administered by trained staff. Interviews were conducted in English, Dagbani, Twi and Hausa. The interview included demographics (age, gender, religion/ethnicity, education, measures of socioeconomic position, location of birth and childhood and location of all residences), information to assess past and current potential exposures to air pollutants (use of tobacco/exposure to environmental tobacco smoke, exposure to indoor cooking using biomass fuels, type of housing, detailed job history), personal and family medical history (diagnosed illnesses, reported symptoms), and other health-related measurements such as weight, height and blood pressure.

Blood and urine sample collection

A temporary structure (clinic) was erected and arranged to allow for the various data collection for each sampling round in both Agbogbloshie and Madina Zongo. Each participant at enrollment and in subsequent rounds provided 20 ml of venous whole blood. Blood was collected by an experienced phlebotomist following sterile procedures via venepuncture of the antecubital fossa into EDTA tubes. In addition, each participant was given a 100 ml, sterile plastic container to provide a urine sample. The urine was then aliquoted into three 10 ml sterile tubes for storage. The blood and urine samples were placed in a cooler with ice blocks and then transported to the University of Ghana after each sampling day, stored at − 80 °C, and later transported on dry ice to the University of Michigan, USA and McGill University, Canada for DNA extraction and metal analysis, respectively.

Elemental analysis

An inductively coupled plasma mass spectrometer (ICPMS Varian; 820MS) was used to detect the levels of blood and urine Cd, Pb and As. The blood and urine samples were digested with nitric acid as detailed by Basu et al. (2011).

All the analytical quality control measures previously described by Srigboh et al. (2016) were used. In summary, all laboratory glassware and plastic were acid-washed (cleaned, soaked for 24 h in 20% nitric acid and rinsed 3 times in Milli-Q water) before use. Accuracy and precision were measured by use of certified reference materials [INSPQ; QM-U-Q1109 (urine); and then QM-B-Q1506 and QM-B-Q1314 (blood)] obtained from the Institut National de Sante Publique du Quebec. Additionally, each batch run contained procedural blanks and replicate runs. For each element analyzed, the theoretical detection limit was calculated as three times the standard deviation of the mean blank value (Supplemental Table 1).

Extraction of DNA from whole blood for LINE-1 methylation

DNA was extracted in the laboratory at Michigan Public Health using the Qiagen DNA Blood Mini Kit, following the manufacturer's recommendations. The purity and quantity of DNA samples were assessed with the Qubit Broad Range Double-stranded DNA assay and Nanodrop Spectrophotometer through the University of Michigan DNA Sequencing Core. The extracted DNA was then stored at – 20 °C until LINE-1 methylation analysis was conducted.

LINE-1 methylation analysis

Sodium bisulfite conversion was performed on 300 ng of extracted genomic DNA using the Qiagen EpiTect Bisulfite Kit per the manufacturer's protocols. PCR amplification was performed for the promoter region of LINE-1 using a previously published assay (Yang et al. 2004). In summary, HotStarTaq Master Mix (Qiagen, Valencia, CA, USA), water, and desalted FWD/RVS primers were combined to create a PCR master mix. Finally, 3 µL of bisulfite-converted DNA was added to each well to bring the final primer concentration to 0.2 mM and the total reaction volume to 30 µL. PCR product quality was confirmed using 2% agarose gels and gel red staining. Following amplification, 12 µL of PCR product was combined with each sequencing primer and analyzed for CpG-specific methylation using the PyroMark MD System (Qiagen, Valencia, CA, USA). Four bisulfite conversion controls (EpigenDX) and four pyrosequencing controls (Qiagen) were prepared at methylation levels of 0%, 30%, 60% and 100%. CpG site-specific methylation percentages (0–100%) were generated for each of the four CpG sites included in the assay. All samples on a plate were rerun if any of the controls failed. Samples were measured in duplicate. The reproducibility of the assay in our duplicate samples expressed as the variation in coefficients was 2%.

Statistical analysis

Statistical analysis was performed with Stata v15.1 (STATA Corp LLC. Texas, USA), and GraphPad Prism v8.3.1 was used to generate graphs. The Shapiro–Wilk test was used to assess the normality of continuous variables. The data are presented as the mean (standard deviation) for normally distributed data or median (interquartile range) for data that were not normally distributed. The nonparametric Mann–Whitney *U* test was used to compare differences across study sites for continuous abnormally distributed data, while the *t* test compared normally distributed data. In the case of categorical variables, the Chi-squared test of association was used for the comparison, while one-way analysis of variance (ANOVA) compared continuous variables (e.g., LINE1) and categorical variables (e.g., job categories). Since Cd and Pb were not normally distributed, they were log-transformed for the bivariate analysis. Pearson correlation analysis was used to assess the correlation between the toxic metals and the average methylation of the four CpG sites (LINE1) and methylation of each CpG site. Multivariable linear regression models were used to explore relationships between DNA methylation and toxic metal biomarker levels in e-waste workers and controls while adjusting for confounders. We set statistical significance at $p < 0.05$.

Results

Demographic characteristics

Overall, participants in the control group were significantly older than their e-waste counterparts (25.4 ± 6.3 vs 32.5 ± 10.4, Table 1). The BMI of the control group (mean $= 23.8 \pm 3.5$) was significantly higher than that of e-waste workers (mean $= 21.6 \pm 2.7$, Table 1). A majority of e-waste workers live within 1 km of the e-waste site and work on an average of 10 h per day for an average of 6 days per week. Regarding education level, 25.3% of e-waste workers had no formal education compared to 13.0% of the controls. Only 16.2% of e-waste workers had secondary school education or higher, compared to 52.2% of the controls. The majority of the e-waste workers (80%) earned between 20–80 Ghanaian Cedi (GHC); the equivalence of 5–15 USD. The prevalence of smoking among the e-waste workers was significantly higher (27.8%) than among the non-e-waste group (12.2%, Table 1).

Heavy metal concentrations in blood and urine in e-waste workers and controls

As shown in Table 2, E-waste workers had lower median blood cadmium (BCd) concentrations than the control group (0.59 µg/L and 0.81 µg/L, respectively, $p = 0.003$), while concentrations of urine Cd (UCd), which is a biomarker of long-term exposure, did not differ between the two populations. Blood lead (BPb) and urine lead (UPb), on the other hand, showed higher significant median concentrations among the e-waste worker group than the control group (76.82 µg/L and 40.25 µg/L, $p = < 0.001$; and 6.89 µg/L and 3.43 µg/L, $p \leq 0.001$, respectively) (Table 2).

The levels of toxic metals in blood and urine were compared to background levels in the US population using the P95 values of the National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention 2019). For blood and urine lead, 99% and 97% of the e-waste workers' samples far exceeded the reference values of 29.3 µg/L and 1.26 µg/L, respectively. In the controls, 74% of the population had BPb higher than 29.3 µg/L, and 94.1% had UPb higher than 1.26 µg/L (Table 2).

LINE1 DNA methylation in e-waste workers and controls

Methylation of four CpG sites of LINE1 was quantified from whole blood samples ($N = 149$). As expected, all CpG sites were heavily methylated. There was no significant difference in LINE1 methylation among the e-waste workers and the non-e-waste workers ($85.16 \pm 1.32\%$ vs $85.17 \pm 1.11\%$, $p = 0.950$). CpG1 showed significantly lower mean methylation among the non-e-waste workers compared to the e-waste workers ($81.70 \pm 1.86\%$ vs $82.48 \pm 2.20\%$, $p = 0.034$), and CpG4 had the highest (91.28%) mean methylation level (Fig. 1).

LINE1 methylation levels of e-waste workers by primary job tasks performed

The main e-waste recycling activities at the Agbogbloshie site include sorting and transporting e-waste materials, the manual dismantling of larger waste types, and the open burning of smaller insulated wires to recover copper and other valuables (Kwarteng et al. 2020). Therefore, the e-waste workers at the Agbogbloshie site in Ghana are categorized into three main groups: collectors/sorters, dismantlers and burners based on their most recent job tasks (Acquah et al. 2019).

The LINE1 methylation level was compared among the different categories of e-waste workers defined by primary job tasks. Overall, e-waste collectors had the lowest mean methylation level than burners and dismantlers (Fig. 2). However, the difference in methylation was not significant (ANOVA, $p = 0.104$).

Table 1 Characteristics of e-waste workers ($n=100$) and controls ($n=51$) enrolled for the study

	Total	E-waste workers	Non e-waste workers	*p* value
Variables	151	100	51	
BMI (kg/m^2), mean (± SD)	22.4 (3.2)	21.6 (2.7)	23.8 (3.5)	**< 0.001**[a]
Age (years), mean (± SD)	27.8 (8.6)	25.4 (6.3)	32.5 (10.4)	**< 0.001**[a]
Workdays/week, mean (± SD)		6.0 (1.0)		
Hours work/day, mean (± SD)		9.7 (4.4)		
Sleep location, *n* (%)		*n* = 97		
On the site		54 (55.7)		
≤ 1 km off-site		35 (36.1)		
> 1 km off-site		8 (8.3)		
Education, *n* (%)	*n* = 145	n = 99	*n* = 46	**< 0.001**[b]
No formal education	31 (21.4)	25 (25.3)	6 (13.0)	
Primary	30 (20.7)	26 (26.3)	4 (8.7)	
Middle/JHS	44 (30.3)	32 (32.3)	12 (26.1)	
Secondary/SHS +	40 (27.6)	16 (16.2)	24 (52.2)	
Marital status, *n* (%)	*n* = 150	*n* = 99	*n* = 51	0.074[b]
Single	73 (48.7)	43 (43.4)	30 (58.8)	
Married	77 (51.3)	56 (56.6)	21 (41.2)	
Income, *n* (%)	*n* = 149	*n* = 99	*n* = 50	0.072[b]
GHC 20–80	120 (80.5)	81 (81.8)	39 (78.0)	
GHC 81–140	10 (6.7)	9 (9.1)	1 (2.0)	
GHC 141–200	8 (5.4)	5 (5.1)	3 (6.0)	
> GHC 200	11 (7.4)	4 (4.0)	7 (14.0)	
Indoor cooking, *n* (%)	*n* = 147	*n* = 98	*n* = 49	**0.009**[b]
Yes	30 (20.4)	14 (14.3)	16 (32.7)	
No	117 (79.6)	84 (85.7)	33 (67.4)	
Alcohol use, *n* (%)	*n* = 148	*n* = 99	*n* = 49	0.086[b]
Never	125 (84.9)	83 (83.8)	42 (87.7)	
Former	6 (4.1)	2 (2.0)	4 (8.2)	
Occasional/regular	17 (11.5)	14 (14.1)	3 (6.1)	
Smoking, *n* (%)	*n* = 146	*n* = 97	*n* = 49	**0.033**[b]
Yes	33 (22.6)	27 (27.8)	6 (12.2)	
No	113 (77.4)	70 (72.2)	43 (87.8)	

SD standard deviation, *N* Total number of participants, *n* (%) frequency(percent frequency)

[a]*p* values obtained by *t* test

[b]*p* values obtained by chi-square test

Relationship between LINE1 methylation and anthropometric and lifestyle factors

The mean methylation of LINE1 was assessed based on anthropometric and lifestyle factors such as age, BMI, smoking, alcohol consumption and indoor status via one-way analysis of variance (ANOVA) (Table 3). LINE1 methylation was not related to age, BMI, smoking or alcohol consumption ($p_{all} > 0.05$). However, the proximity of e-waste worker residence in relation to the e-waste site was associated with LINE1 methylation ($p = 0.019$). A Tukey post hoc test revealed that LINE1 methylation was statistically lower in workers who lived within 1 km of the e-waste site than those who lived on-site ($p = 0.034$).

Relationship between LINE1 methylation and blood and urine levels of Cd and Pb

Bivariate analyses (Pearson correlation) showed a trend toward negative correlations between the log-transformed toxic metals and LINE1 methylation, even though the correlations were not significant (Table 4). No correlation was observed between LINE1 and the specific CpG sites.

LINE1 methylation and exposure to toxic metals: multiple linear regression

Associations between LINE1 DNA methylation and body burden of toxic metals were assessed using a single metal

Table 2 Cadmium and lead concentrations in blood and urine of e-workers and controls

Toxic metals (µg/L)	Total	E-waste	Controls	*p*
BCd	*n* = 150	*n* = 100	*n* = 50	
Mean ± SD	0.80 ± 0.59	0.73 ± 0.55	0.93 ± 0.64	0.003
Median (IQR)	0.66 (0.44)	0.59 (0.43)	0.81 (0.55)	
BCd > 1.17, *n* (%)	21 (14)	11 (11)	10 (20)	
BPb	*n* = 150	*n* = 100	*n* = 50	
Mean ± SD	75.12 ± 58.42	92.35 ± 63.69	40.67 ± 19.12	< 0.001
Median (IQR)	518.95 (44.01)	76.82 (49.37)	40.25 (17.45)	
BPb > 29.3, *n* (%)	135 (90.67)	99 (99)	37 (74)	
UCd	*n* = 149	*n* = 98	*n* = 51	0.878
Mean ± SD	0.62 ± 0.98	0.68 ± 1.18	0.50 ± 0.35	
Median (IQR)	0.40 (0.45)	0.38 (0.56)	0.42 (0.44)	
UCd > 0.77, *n* (%)	31 (20.8)	22 (22.4)	9 (17.6)	
UPb	*n* = 149	*n* = 98	*n* = 51	< 0.001
Mean ± SD	6.49 ± 5.03	7.76 ± 4.87	4.06 ± 4.45	
Median (IQR)	5.05 (4.98)	6.89 (5.22)	3.43 (2.38)	
UPb > 1.26, *n* (%)	145 (97.3)	97 (99)	48 (94.1)	

p value estimate from the Wilcoxon rank-sum (Mann–Whitney) test

p values < 0.05 were considered significant

IQR interquartile range

Source of reference values USA, NHANES, Survey 2011–2016 (U.S. Department of Health and Human Services—Centers for Disease Control and Prevention 2019)

linear regression and mutual linear regression models, adjusting for potential confounding factors that may influence methylation status (age, BMI, smoking, alcohol consumption and indoor use of biomass fuel for cooking).

In the e-waste worker group, the single metal linear regression analyses found no significant association between LINE1 and any of the metals, ($p_{all} > 0.05$). In the control group, only BPb showed significant negative association with LINE1 methylation ($\beta_{BPb} = -0.027$, 95% CI −0.045, −0.010, $p = 0.003$) (Table 5). The mutual regression analysis results were similar to that of the single metal analysis with a marginal increase in the association between BPb and LINE1 methylation only in the control group ($\beta_{BPb} = -0.028$, 95% CI −0.045, −0.011, $p = 0.002$). Noteworthy, we found a marginal association between BPb and LINE1 methylation in the e-waste exposed group in the multiple metals analysis ($\beta_{BPb} = -0.005$, 95% CI −0.011, −0.000, $p = 0.058$) (Table 5).

Discussion

Informal e-waste recycling activities generate a considerable amount of air pollutants, including toxic metals, some of which are carcinogenic (Alabi & Bakare 2017). Several studies (Goodrich et al. 2013; Li et al. 2011, 2013; Wen et al. 2016) have reported associations between occupational heavy metal exposure and DNA methylation; however, there are few studies, if any, that have looked at exposure levels and DNA methylation among informal e-waste workers.

Our study showed that lead levels (BPb and UPb) were significantly higher in e-waste workers than in controls. This finding is consistent with other biomonitoring studies among e-waste workers at Agbogbloshie (Wittsiepe et al. 2017) and teenage e-waste scavengers in Nigeria (Alabi et al. 2019), where BPb and serum Pb levels were consistently higher in the exposed group than in the controls, suggesting e-waste recycling activities as a critical contributor to the elevated Pb levels. Contrary to our expectation, BCd was significantly higher among the controls.

In this study, the control population had generally high Cd and Pb levels in blood and urine compared with the P95 values of the NHANES survey (Centers for Disease Control and Prevention 2019). The high concentration of toxic metals in the unexposed group could be attributed to the reliance on solid fuels for cooking, a significant source of ambient or outdoor air pollution. The Health Effects Institute (HEI)—Ghana working group estimated that residential sources (including household cooking, lighting and heating) contributed approximately 65% of the total national primary $PM_{2.5}$ in Ghana, followed by transport and road dust (13.9%) (Health Effects Institute 2019). Additionally, this study's control group was composed of population-based subjects, most of whom live and work near a busy highway with frequent vehicular traffic and may be exposed to traffic-related air pollutants. Furthermore, drinking water

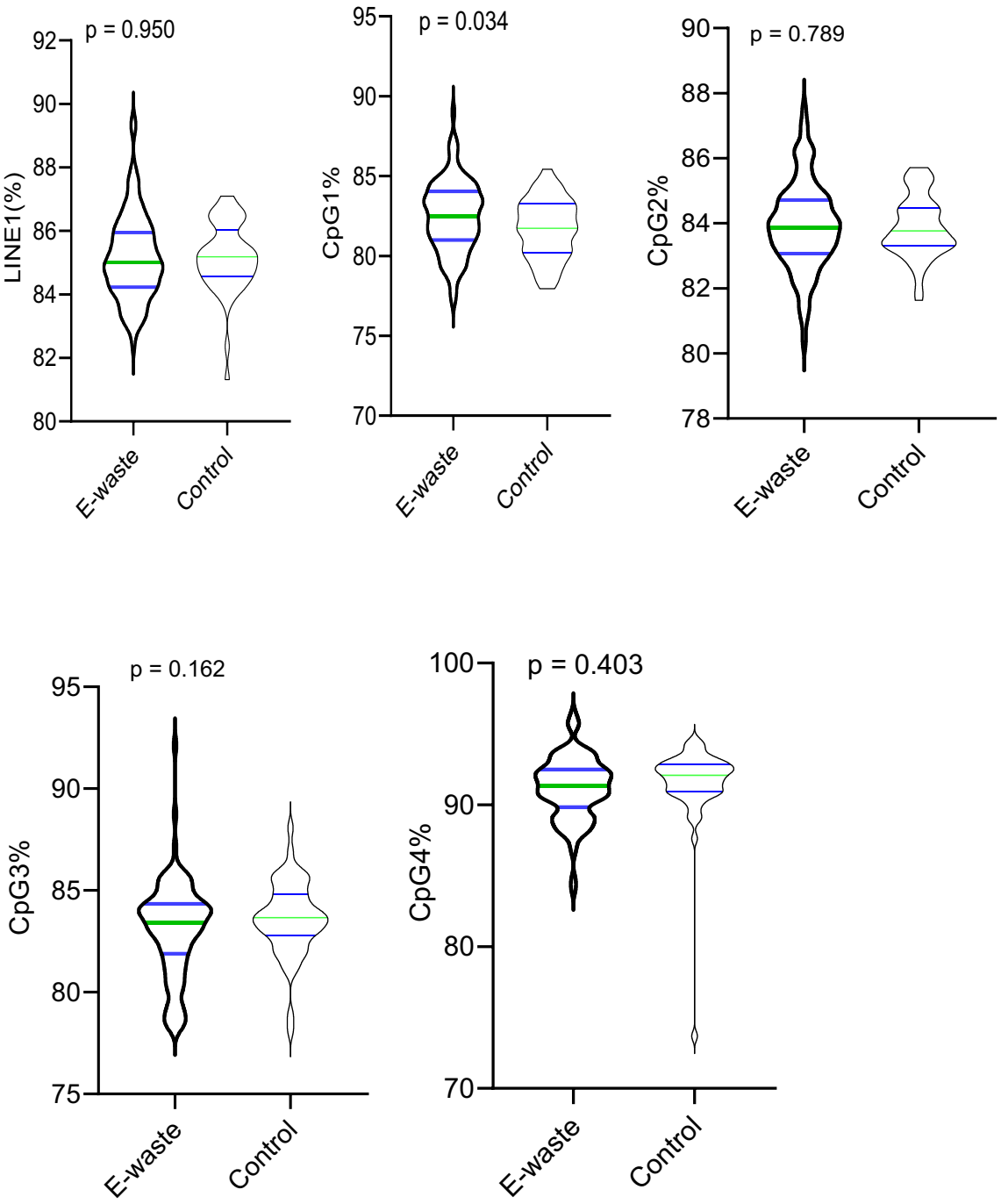

Fig. 1 violin plots. The violin plots present the distribution of the individual methylation of LINE1 and specific CpG sites of LINE1 in e-waste workers and controls. *P* values were calculated by *t* test. The green line represents the median, and the blue lines represent the interquartile range.

could be a major source of toxic metal exposure in Ghana. For example, Asante et al. (2012) estimated the levels of trace element (T.E.s) concentrations in tap water in Accra, Ghana, and found significant variations in heavy metal concentrations such as barium (Ba) (GM; 36 µg/l), manganese (Mn) (GM; 19.5 µg/l), zinc (Zn) (GM; 18.7 µg/l) and iron (Fe) (GM; 18 µg/l), among others, even though the samples were taken from the same water treatment source. The use of metal (galvanized iron) pipes for water distribution in Ghana and corroded household plumbing systems were reported as possible sources of toxic metals found in tap water (Asante et al. 2012). Further, the high body burden of Cd in both

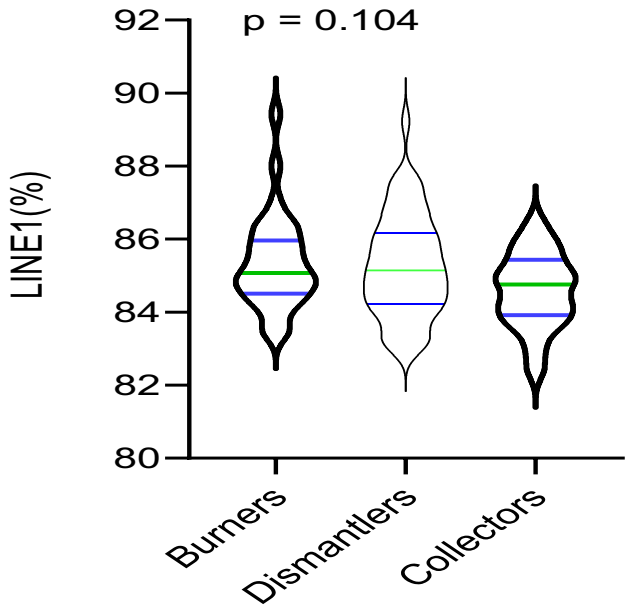

Fig. 2 LINE1 methylation across primary job-tasks performed by e-waste workers; collectors had the lowest mean methylation levels than burners and dismantlers. The green bar indicates median methylation, and the blue bars represent the interquartile range

the e-waste workers and controls could be attributed to cadmium exposure from contaminated food other than from occupational exposure. Tubers of yam and potatoes, rice and vegetables are staple foodstuffs for this population and are mostly farmed in contaminated soil (Bortey-Sam et al. 2015); therefore, exposure to Cd in this population could be via the ingestion of these contaminated foodstuffs.

Our findings show that LINE1 was heavily methylated in the whole blood of e-waste workers (mean ± SD: 85.2 ± 1.3%). Overall, there was no significant difference in LINE1 methylation between the e-waste workers and controls in this study. Even though CpG1 showed a significant difference between the e-waste workers and controls, this was not sufficient to influence the overall difference in LINE1 methylation between the groups. This is consistent with Ghosh et al. (2017) findings, where no significant difference in LINE1 methylation was observed between workers exposed to multi-wall carbon nanotubes and controls. The non-significant difference in LINE1 methylation between our study populations may be attributable to the choice of the control group in this study since the categorization of hyper- or hypomethylation is dependent on the methylation levels of the comparator group (Phetliap et al. 2018). For example, the differences in age between the e-waste workers and controls could affect our comparison of their methylation levels. However, the average age of the e-waste workers (25.4 ± 6.3 years) and controls (32.5 ± 10.4 years) are not in the category of vulnerable window including the elderly

Table 3 Relationship between LINE1 methylation and anthropometric and lifestyle factors

Variable	LINE1 methylation			
	Total ($n=151$)	E-waste workers ($n=100$)	Non e-waste workers ($n=51$)	p value
Age (years)				
≤20	85.3 (1.1)	85.2 (1.2)	85.4 (0.6)	0.817
21–30	85.1 (1.4)	85.1 (1.4)	85.3 (1.3)	0.520
31–40	85.1 (1.2)	85.3 (1.3)	84.8 (1.2)	0.3995
>40	85.3 (1.2)	85.73 (2.0)	85.2 (1.0)	0.541
	$p=0.885$	$p=0.803$	$p=0.697$	
Smoking				
Yes	85.2 (1.2)	85.2 (1.3)	85.1 (0.7)	0.805
No	85.2 (1.3)	85.2 (1.3)	85.2 (1.2)	0.892
	$p=0.914$	$p=0.830$	$p=0.832$	
Alcohol intake				
occasional/ regular	85.4 (1.4)	85.4 (1.5)	85.6 (1.3)	0.797
former	85.2 (1.6)	84.2 (2.5)	85.7 (1.2)	0.339
never	85.2 (1.2)	85.2 (1.3)	85.1 (1.1)	0.744
	$p=0.675$	$p=0.471$	$p=0.481$	
BMI (kg/m^2)				
Low weight	84.8 (1.1)	84.6 (1.2)	85.2 (0.5)	0.492
Normal weight	85.2 (1.3)	85.2 (1.3)	85.2 (1.1)	0.796
Overweight	85.3 (1.2)	84.9 (1.3)	85.5 (1.1)	0.307
Obesity	84.5 (1.3)	85.7 (0.0)	84.2 (1.3)	0.364
	$p=0.363$	$p=0.529$	$p=0.244$	
Sleep location				
On-site	–	85.4 (1.4)	–	–
≤1 km off-site	–	84.7 (1.1)	–	–
≥1 km off-site	–	85.8 (1.4)	–	–
	–	$p=$**0.019**	–	–
Indoor cooking				
Yes	85.1 (1.3)	85.3 (1.5)	84.9 (1.1)	0.370
No	85.2 (1.2)	85.2 (1.3)	85.3 (1.1)	0.574
	0.733	$p=0.635$	$p=0.252$	

Body Mass Index (BMI) according to World Health Organization (WHO) parameters: low weight (≤18.5 kg/m^2); normal weight (>18.5 kg/m^2 and ≤24.9 kg/m^2); overweight (>24.9 kg/m^2, and ≤29.9 kg/m^2), and obesity (≥30 kg/m^2). p values were obtained by the ANOVA test

(Bollati et al. 2009), and children and adolescents (Burris et al. 2011), where environmental exposures greatly affect the epigenome. No significant relationship was observed between age and LINE1 methylation in both the e-waste workers and controls. This conforms to other studies that did not report differences between age and LINE1 methylation (Benitez-Trinidad et al. 2018; Marques-Rocha et al. 2016; Zhu et al. 2012). In addition, indoor and outdoor urban air pollution, especially in the form of particulate matter (PM),

Table 4 Pearson correlation coefficients between LINE1 and specific CpG site methylation and log-transformed blood and urine Cd and Pb

Toxic metals	LINE1		CpG site1		CpG site2		CpG site3		CpG site4	
	R	*p*	*R*	*p*	*r*	*P*	*R*	*p*	*R*	*P*
BCd	− 0.06	0.476	− 0.00	0.996	− 0.11	0.176	− 0.11	0.171	0.04	0.668
BPb	− 0.14	0.085	0.04	0.599	− 0.13	0.128	− 0.15	0.076	− 0.13	0.104
UCd	− 0.14	0.089	− 0.14	0.088	− 0.06	0.498	− 0.06	0.445	− 0.08	0.333
UPb	− 0.03	0.729	0.11	0.200	0.02	0.833	− 0.01	0.940	− 0.13	0.117

R correlation co-efficient, *p* p value

Table 5 Single metal linear regression models and multiple metals linear regression models for LINE1 in e-waste workers ($n=100$) and controls ($n=51$)

Predictors, µg/L	E-waste workers		Controls	
	LINE1			
	β (95% CI)	*p* value	*β* (95% CI)	*p* value
Models[1]				
Blood Cd	− 0.112 (− 0.804, 0.581)	0.750	0.008 (− 0.555, 0.572)	0.975
Urine Cd	− 0.055 (− 0.295, 0.185)	0.651	− 0.408 (− 1.465, 0.650)	0.440
Blood Pb	− 0.003 (− 0.007, 0.002)	0.213	− 0.027 (− 0.043, − 0.010)	**0.003**
Urine Pb	0.014 (− 0.046, 0.074)	0.644	− 0.037 (− 0.115, 0.042)	0.354
Models[2]				
Blood Cd	0.072 (− 0.663, 0.806)	0.846	− 0.053 (− 0.564, 0.458)	0.835
Urine Cd	− 0.166 (− 0.451, 0.118)	0.249	− 0.539 (− 1.558, 0.480)	0.290
Blood Pb	− 0.005 (− 0.011, 0.000)	0.058	− 0.028 (− 0.045, − 0.011)	**0.002**
Urine Pb	0.071 (− 0.013, 0.154)	0.095	− 0.021 (− 0.096, 0.054)	0.580

Models[1] = single metal linear regression models

Models[2] = multiple metals linear regression models

All models adjusted for age, BMI, smoking status, alcohol intake, and indoor biomass fuel use for cooking. Bold *p* values are statistically significant

generated from the widespread use of biomass fuelwood as an energy source for cooking is a major public health concern in Ghana (Cobbinah et al. 2017). This and other sources, such as vehicular emissions, exposes a large proportion of the urban population to PM, from which our control group for this study was recruited. Therefore, our control group is substantially exposed to a high concentration of ambient air pollution, which could explain the lack of difference in LINE1 methylation in our study since traffic-related particles ($PM_{2.5}$) altered the level of LINE-1 methylation in blood cells (Baccarelli et al. 2009).

Workers at the e-waste recycling site in Ghana are involved in multiple tasks since job titles and task protocols are not present, and previous studies (Feldt et al. 2014; Wittsiepe et al. 2015), as well as this study, derived job categories based on workers' self-report. Regarding the job tasks performed by the e-waste workers, collectors had lower LINE1 methylation, even though the difference was not statistically significant. This could be attributable to job misclassification, which may conceal a significant difference in health effects (Laskaris et al. 2019). The use of wearable cameras proposed by Laskaris et al. (2019) can significantly minimize task misclassification and improve occupational exposure assessment among informal sector workers. E-waste collectors often travel off-site within communities (primarily by foot, bicycle or tricycle) to purchase or scavenge e-waste materials (Acquah et al. 2019; Laskaris et al. 2019) and may, therefore, be exposed to other sources of pollutants (including vehicular emissions and road dust) outside the e-waste recycling site. The decline in LINE1 methylation among collectors could, therefore, be attributed to the higher exposures due to multiple exposure sources.

Our single metal multivariable regressions showed that BPb was associated with decreased LINE1 methylation in only the controls. This association was marginally stronger when Cd was adjusted for in the multiple metals regression analysis. The associations between BPb and LINE1 methylation among only the controls could be attributed to the significant concentration of BCd in the controls. Cadmium could add to the burden of Pb on DNA methylation, as observed in our multiple metals regression analysis. Our finding is consistent with previous occupational exposure studies in China (Li et al. 2013) and Brazil (Devóz et al. 2017). In the Chinese study, methylation levels of LINE1 were assessed among battery workers exposed to Pb ($n=53$) and a healthy control group ($n=57$), and they reported a

significant decrease in LINE1 methylation among the Pb-exposed group (Li et al. 2013). Among workers in an automotive factory in Brazil, there was a negative association between Pb exposure and global DNA methylation (Devóz et al. 2017). Lead is reported to alter DNA methylation by decreasing the activities of DNA methyltransferase (DNMT), the enzyme that regulates the DNA methylation reaction by catalyzing methyl groups (CH_3) through SAM to DNA, which initiates DNA hypomethylation (Sanchez et al. 2017). However, a recent epigenome-wide study did not show any significant methylation changes in DNMTs among workers occupationally exposed to Pb, suggesting that other genes may mediate Pb-induced DNA methylation changes (Zhang et al. 2019).

In the e-waste worker group, a weak association was observed between BPb and LINE1 methylation ($p = 0.058$) only in the multiple metals regression model. The weak association in the e-waste workers suggests that Cd adds to the burden of Pb on LINE1 methylation in both the e-waste workers and controls. Cadmium is both an occupational and environmental toxicant and is established as a carcinogen that likely acts via epigenetics mechanism due to its weak mutagenicity (Martinez-Zamudio and Ha 2011; Takiguchi et al. 2003). Few studies have reported on the associations between environmental Cd exposure and global DNA methylation with acute exposure associated with increased methylation, whereas chronic exposure is associated with decreased methylation (Martinez-Zamudio and Ha 2011). For example, Hossain et al. (2012), reported an inverse association between UCd, which is a marker of chronic exposure and LINE1 methylation in women exposed to low levels of environmental Cd. The mechanism by which Cd influence DNA methylation is not fully understood and is reported to alter DNA methyltransferases and ten–eleven translocation (TET) enzyme activities (Ruiz-Hernandez et al. 2015).

There are some limitations to this study. First, exposure in the informal e-waste recycling sector is to a complex mixture of chemicals, including PAHs and other persistent organic pollutants (POPs), which may also alter DNA methylation, data of which were not included in this study. Second, other domains of environmental exposure, such as nutrition and psychosocial stress, may result in epigenetic modifications that may contribute to an increased risk of disease (Thayer and Kuzawa 2011), data of which were not considered for this study. Third, attempts to match the age of controls to e-waste workers were made during recruitment, but consent to participate was higher among the older controls. This issue was addressed by adjusting for age in all of our analysis models. Finally, DNA was extracted from circulating blood that does not represent a potential target organ such as bladder, pancreas, or stomach. However, the use of circulating blood DNA as a proxy for specific organ health status is reported (Barchitta et al. 2014; Ponomaryova 2021).

The present study's strength was that we examined two toxic metals concurrently using two types of biological media (blood and urine) for the exposure assessment. Additionally, the present study benefitted from high-quality protocols for recruiting participants, conducting interviews, collecting biological samples and laboratory analyses.

In conclusion, the high internal concentration of toxic metals in the control group in this study suggests that toxic metals exposure is a nationwide problem in Ghana. However, we found that e-waste workers tend to have a higher concentration of Pb in particular, which was associated with global DNA hypomethylation, as shown in LINE1 methylation in both e-waste workers and controls. This may serve as an early epigenetic marker that mediates the adverse effects of Pb exposure. In addition, e-waste collectors had decreased LINE1 methylation levels compared to the other categories of workers. To the best of our knowledge, this is the first study that examined this population in an epigenetic context. Since global methylation provides important preliminary information about genome stability, further epigenetic epidemiologic studies are needed using candidate genes and other epigenetic markers, such as histone modifications, to provide a comprehensive understanding of specific pathways through which toxic metals exert their toxic effects, especially among unprotected informal sector workers.

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Author contributions Conceptualization: II, TGR, JNF, LSR and JA-M; Methodology: II, LSR, KRZ, JNF and JA-M; Formal analysis and investigation: II, DD, TGR, JNF, JA-M, NB, TPA, LSR, and KRZ; Writing—original draft preparation: II; Writing—review and editing: II, LSR, KRZ, JNF, JA-M, and TPA; Funding acquisition: TGR, SB, NB, and JNF; Resources: TGR, JNF Supervision: JNF, TGR, JA-M, and LSR. All authors read and approved the final manuscript.

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Availability of data and materials The datasets generated and/or analyzed during the current study are not publicly available due to privacy reasons but are available from the corresponding author on reasonable request.

Declarations

Ethics approval The study was conducted after receiving ethical clearance from the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana (protocol identification number CHS-ET/M.4-P 3.9/2015–2016).

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no competing interests.

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