Urinary 8-OHdG level is not affected by geography and trace elements in nail of residents of Addis Ababa: It is shaped by interactions between different social factors

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ABSTRACT

The objective of this study was to evaluate the association between exposure to heavy metals and oxidative DNA damage among residents living in the potentially more polluted downstream (Akaki-Kality) area of Addis Ababa, in comparison to the upstream area (Gullele). For this, 8-hydroxy-2′-deoxyguanosine (8-OHdG) was used as a biomarker for oxidative DNA damage and heavy metals (Fe, Zn, Mn, Cu, Ni, Cr, Pb, As) as indicators of exposure. The concentrations of heavy metals in nails were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES), and 8-OHdG in urine using Enzyme-Linked with Immunosorbent Assay (ELISA), from 95 residents of the two areas, upstream and downstream. The urinary 8-OHdG concentration was not significantly different (p = 0.05) between the two Sub-Cities, with mean of 18.50 ± 4.37 ng/mg Creatinine in Akaki-Kality and 17.30 ± 5.83 ng/mg Creatinine in Gullele. Also, there were no statistically significant (p = 0.05) difference among the different demographic groups according to gender, age, educational status, body mass index or habit of alcohol consumption. However, the interactions of sex with age, sex with alcohol consumption and alcohol consumption with education were found to affect the urinary 8-OHdG levels of residents of the two Sub-Cities. The mean concentrations (µg/g) of the elements were 488 and 1035 for Fe, 106 and 251 for Zn, 13.0 and 31.2 for Mn, 5.23 and 6.63 for Cu, 11.2 and 7.39 for Ni, 2.23 and 2.02 for Cr, 0.09 and 0.63 for Pb; and 0.16 and 0.25 for As, in nail samples from Akaki-Kality and Gullele, respectively. The determined concentrations of the heavy metals in nails were not significantly associated (p = 0.05) with the corresponding urinary levels of 8-OHdG. Hence, the observed 8-OHdG might have been caused by environmental exposure to toxic substances other than the analyzed heavy metals.

1. Introduction

Addis Ababa is the fastest-growing city of Ethiopia both in terms of population growth and rate of urbanization; it is also the center of Ethiopia’s political and financial administration. As a result, it attracts several industries [1], which play pivotal roles in the development of the capital city and country. Of the various industries, tannery industries generate large quantities of waste which cause adverse impacts on the environment. Currently, the Ethiopian tannery industry plays a prominent role in the advancement of the sector in Eastern and Southern

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Africas [2]. The quality of water bodies in Addis Ababa City has deteriorated, which is ascribed to the large amounts of unrestricted discharge of effluents from industrial, domestic and municipal wastes [3,4].

Effluents from tannery, other industries, domestic and commercial centers contain high levels of chemicals such as dissolved and suspended solids, acids, dyes, various organic and inorganic substances, and heavy metals including Cr, Zn, Cu, Mn and Pb [5,6]. Exposure to heavy metals from dumping of hazardous material from industries into rivers in a number of fast-growing cities of developing countries like Ethiopia, pose pollution problems and health hazards [7,8].

Residents living around Akaki River are exposed to these toxicants as a result of using wastewater for irrigation and ingestion of vegetables grown alongside of the polluted river, and through inhalation and dermal contact pathways [9,10]. Since heavy metals are non-degradable, have long half-lives; and can accumulate in the human body, they undergo redox reactions to produce reactive oxygen species (ROS) like hydroxyl radicals (OH·), superoxide anion (O₂·), singlet oxygen (¹O₂) and hydrogen peroxide (H₂O₂) in biological systems [11]. These ROS are capable of disrupting the activity of many proteins involved in reproductive and endocrine systems, altering the patterns of expression of multiple genes, and increasing oxidative DNA damage, lipid peroxidation and other effects [12-17].

A wide range of substances are produced during DNA oxidative damage including bases and sugar modifications, covalent cross links and single and double strand breaks. Among the diverse oxidative DNA lesions, 8-hydroxy-2-deoxyguanosine (8-OHdG) is the most abundant modified base that has attracted research attention as it causes G-to-T transversions [18], which lead to mutagenesis. This compound is mainly produced between hydroxyl radicals and guanosine at the C-8 position in DNA [19] and helps to examine DNA oxidative stress from metals exposure [20,21]. Hence, measurement of 8-OHdG in urine is generally considered as a common biomarker for assessing DNA damage caused by occupational metal exposure [14,22,23]. Collection of urine samples is relatively simple, and non-invasive; it is stored with low biological hazard, no pretreatment is needed prior to storage, only a small volume is required, and 8-OHdG can be detected precisely using HPLC with electrochemical or mass detector or immunochromatocassay. Besides, urinary 8-OHdG is not affected by diet or cell turnover [11,24].

Oxidative DNA damage can occur at both the nuclear and mitochondrion levels [25] and propensity to damage increases with age [26]. While oxidative damage directly influences DNA mutations, this may only manifest in humans when sufficient damage has occurred to affect apoptosis and replicative cell function. Conditions known to be associated with oxidative DNA damage include disorders of neurodegeneration such as Alzheimers disease, Parkinsons disease, autoimmune diseases, diabetes mellitus and cardiovascular disease and stroke and a number of cancers such as lymphoblastic leukemia, breast cancer, colorectal cancer, renal cell carcinoma, hepatocellular carcinomas and lung cancer to name a few [27]. According to the Global Burden of Disease data (2019), the 10 most common causes of death in Ethiopia are cardiovascular disease, cancers, maternal and neonatal death, respiratory infections, musculoskeletal disorders, mental health and neurological illness, diabetes and kidney disease and chronic respiratory disease and several of these may be contributed to at least in part by oxidative DNA damage [28].

Several studies have shown that heavy metal exposure can cause damage to sperm DNA and dramatically increase 8-OHdG levels in urine [29,30]. Researchers have also found significant positive correlation between urinary concentrations of Mn, As, Cu and Cd and levels of 8-OHdG in samples from college students in Guangzhou, China [11,31]. It has also been reported that welders exposed to heavy metals have higher urinary 8-OHdG concentrations than that of a control community. Similarly, the urinary levels of 8-OHdG in people living around an electronic waste dismantling area in Qingyuan City, China, were positively correlated with urinary levels of different metals [32].

Human biomonitoring is commonly used to assess exposure to toxic pollutants [33,34]. In contrast to the various bio-tissues used for biomonitoring, such as urine and blood, the use of nails offers an advantage as they can experience a longer time of bio-indicator of exposure to toxic elements [35]. This is because elements are steadily incorporated into nail over time, as it is an excretory system. In addition, the nail has lower fluctuation in element levels due to changing body metabolic activities [36].

Literature sources (Appendix A, Supplementary material), have indicated that exposure to trace elements as a result of river pollution in the downstream reaches of the Akaki catchment, in Addis Ababa, are higher than upstream reaches. For instance, concentrations of As [37,38], Fe [39], Cu [37,39,40], Mn [37-39], Zn [37,40,41], Cr [37-39,42], Pb [39-41] and Ni [37,38] in river and irrigation waters have exhibited this trend in the Akaki catchment. Even though residents in the downstream of Akaki catchment are potentially more exposed to heavy metals, and hence a higher risk of oxidative DNA damage, than those in the upstream, no study has investigated the link between toxic elements and 8-OHdG levels in bio-samples from the residents. Therefore, this study tested the hypothesis that the residents of Addis Ababa in the downstream (Akaki-Kality Sub-City) of the Akaki catchment to have a higher level of urinary 8-OHdG than the upstream (Gullele Sub-City). This research looked to evaluate: (1) variations in urinary 8-OHdG levels of the residents with socio-demographic factors: gender, age and educational status, as well as personal habits: alcohol consumption and physical condition i.e., body mass index; (2) variations in urinary 8-OHdG levels between residents of Akaki-Kality and Gullele; (3) association of the potentially toxic elements in nail with 8-OHdG level in urine.

2. Materials and methods

2.1. Description of the study area and population

This study was designed to investigate and compare the extent of exposure to toxic metals by residents living in the downstream area (Akaki-Kality) with those living in upstream (Gullele) area of Addis Ababa. Addis Ababa is subdivided into 11 Sub-Cities: Gullele, Yeka, Addis-Ketema, Arada, Kolfe-Keranio, Lideta, Kirkos, Bole, Nefas Silk-Lafto, Akaki-Kality and the recently created Sub-City of Lemi-Kura. Akaki-Kality and Gullele were selected as representative of the most upstream and downstream areas of the Akaki catchment in Addis Ababa (Fig. 1).

Big Akaki (BAR) (right in Fig. 1) and Little Akaki (LAR) (left in Fig. 1) are the two major rivers that flow through Addis Ababa from North to South. However, these rivers are exposed to severe pollution from untreated domestic and industrial wastes. Little Akaki River, in particular, is more exposed to pollution as it traverses through highly populated and dense industrial areas of the city that hosts tanning, textiles, battery and paints, workrooms, food and beverages, pharmaceutical, marble, glass, shoe, oil and soap factories, among others [9].

The sample size used in the study was calculated based on a formula provided by Yamane [43] for a simple random sample. The formula uses 95 % confidence level and with 50 % estimated proportion of an attribute that is present in the population:

\[
 n = \frac{N}{1 + \frac{N(e)^2}{N - 1}}
\]

where \( n \) is the sample size, \( N \) is the population size, and \( e \) is the level of precision. When this formula is applied for a population size of over 1,000,000, in Gullele and Akaki-Kality Sub-Cities of Addis Ababa, and setting the level of precision at \( 10 \% \), a sample size of 100 is obtained as follows:

\[
 n = \frac{1000000}{1 + 1000000(0.1)^2} = 99.99
\]
Accordingly, a total of 95 households, 53 in Akaki-Kality and 42 in Gullele, participated in the study. The Sub-Cities were stratified into smaller administrative districts, and the numbers of participating households were evenly allocated to the districts.

Following written consent and an ethical approval obtained from Ethical Review Board of Addis Ababa University, individuals invited to the study were based on purposive sampling aimed to recruit an adult (>18 years) from every household. Nail and urine samples were collected from one volunteer per household aimed at achieving gender balance between men and women. In addition, an individual questionnaire gathering information on employment, gender, education level, body mass index (BMI), alcohol consumption behavior, occupation, smoking and health history was collected from the volunteers. Factory workers, people with history of cancer and those resided less than three years in the area were excluded from the study. The data on occupation was too diverse to summarize and draw any useful conclusion out of it, while the data on smoking showed the participants comprised only 2% smokers, and hence these two parameters were not further used in the analysis.

2.2. Chemicals, standards and solutions

Hydrochloric acid (37%, ACS reagent, Germany), Triton X-100 (0.5% v/v, ACS reagent, Germany), argon (99.99%), hydrogen peroxide (30%, ACS reagent, Germany), nitric acid (65%, ACS reagent, Germany), acetone (99.9%, ACS reagent, Germany) and 8-OHdG (Biobyt, UK) were used. High-purity deionized water (18.2 MΩ/cm) was used for dilution of standard stock solutions and preparing samples throughout the analysis for inductively coupled plasma optical emission spectrometry (ICP-OES). A series of standard solutions of 2.5, 5.0, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/L were prepared from 100 mg/L stock solutions of each element to calibrate the ICP-OES. The calibration curves showed good linearity with regression coefficients greater than 0.998 for all of the quantified elements.

2.3. Sample collection, preparation and analysis

2.3.1. Nail samples

Participants were requested to wash their hands and feet with medicinal soap and water before the samples were collected. Then the finger and toe nails of participants were clipped and placed in airtight plastic vials.

In the laboratory, the nail samples were firstly scratched manually with ceramic knife to remove any surface contamination. Each sample was immersed in non-ionic detergent solution overnight, shaken by auto-shaker for 20 min and washed with tap water. Subsequently, the samples were washed three times in an auto-shaker for 20 min in acetone and again shaken for another 20 min with Triton X-100 (0.5%, v/v). Then, the samples washed with deionized water. Finally, they were dried in an oven overnight at 70°C.

The dried samples were digested according to Dessie et al. [35]. Briefly, a 100 mg sample was transferred in to a conical flask containing 6 mL of a mixture of HNO₃ and HCl (3:1, v/v). The flask was covered with watch glass and kept undisturbed for 30 min at room temperature. The sample was then digested at 80°C near dryness and cooled to room temperature. To the dried sample, 2 mL of concentrated HNO₃ was added and the residue after heating was dissolved with deionized water. Finally, the digested solution was filtered using a 0.2 µm filter, adjusted to 8 mL with deionized water and stored at 4°C until analysis. Blank samples, for the estimation of detection limits of the method, were also prepared following the same procedure used for the digestion of samples.

2.3.2. Determination of elements in nail samples

The concentration of elements in the digested nail samples were determined using ICP-OES. Before analysis of the samples, the instrument was conditioned for 30 min and optimized by running the daily performance check solution. The instrument operating conditions such as the nebulizer flow rate, the position of the torch, radio frequency (RF) power and interference correction were verified. Each sample was
analyzed in triplicate and the concentrations were determined as an average of replicates. When sample dilutions were necessary, they were performed by pipetting the required volume of sample into a sample tube and combined with the required volume of ultra-pure water, capping and inverting several times in order to avoid signals that were too high for the detector to measure adequately. Finally, potentially toxic elements in nails were quantified based on the calibration curve.

2.3.3. Urine samples

A 50 mL morning urine sample was collected from the participants in polyethylene bottles, which were previously cleaned and treated with HNO₃. The samples were transported to laboratory immediately in an ice box and stored at −80 °C until analysis.

2.3.4. Determination of 8-OHdG in the urine samples

Urine 8-OHdG level was determined by competitive ELISA (Enzyme-Linked with Immunosorbent Assay) as previously reported [45,46]. A blank well without any solution was set and 50 µL of urine samples were added to each well in a duplicate manner. Antibody (50 µL) was added to each well (but not to the blank well) immediately and shaken gently for 60 s followed by incubation at 37 °C for 30 min in a shaker incubator. Then the plates were washed three times with wash buffer (250 µL) and the remaining wash buffer was removed by aspirating or decanting. Horseradish peroxidase (HRP) - conjugate (100 µL) was added to each well, covered with adhesive strip, incubated at 37 °C for 30 min and washed five times as described previously. Next, 90 µL of tetramethyl benzidine (TMB) substrate was added to each well followed by incubation at 37 °C for 20 min. Finally, the reaction was stopped by adding 90 µL stop solution and the optical density was determined using microplate reader set to 450 nm. The optical imperfection in the plate was corrected by subtracting readings at 570 nm from the readings at 450 nm. Finally, the concentrations of 8-OHdG in the urine samples were interpolated from the standard curve (Fig. 2), and all statistical tests were performed using interpolated values. The standard curve for human 8-OHdG was generated using Graph Pad Prism version 9.01, and the equation used was a fourth order polynomial least squares fit with R² = 0.9999.

2.4. Socio-demographic data collection

Socio-demographic and related data, including age, sex, education, alcohol consumption and BMI were collected from all the study participants. The data were collected after receiving ethical clearance from Ethical Review Board of Addis Ababa University. The information was obtained after completing validated structured questionnaire (translation to the mother tongue of the participants). Experienced data collectors were involved in information and data collection. Individuals were fully informed of the process and confirmed they fully understood the aim of the research and signed informed written consent to participate.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS statistical package (IBM, version 23). Homogeneity of variance and normality of the distribution of 8-OHdG concentrations and potentially toxic elements in samples were tested by using Levene statistic and Kolmogorov-Smirnov Statistic with Lilliefors’ Significance, respectively. Non-parametric tests, based on Kruskal-Wallis one-way ANOVA and Mann-Whitney U independent two sample test, were used to evaluate the presence of significant differences among groups of samples. Box and whiskers plots were used to visualize distribution among samples. In the plots outliers are identified as cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box, while extremes showed cases with values more than 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range. The presence of significant association between 8-OHdG and potentially toxic elements was assessed from Pearson correlation coefficients. Differences were significant when p < 0.05. Principal component analysis was used to visualize sample trends.

2.6. Quality assurance

Accuracy of the methods were assessed by spiking the samples in every batch of the sample digestion and analysis. A known amount of the element of interest, which is 100 % of the amount found in the sample, was added to the nail sample and subjected to the same digestion and analysis procedure utilized for the samples. The percentage recoveries (Table 1) ranged from 94.7 % (As) to 110 % (Zn), ensuring that the method was accurate for determining each element. The precision of the analytical method was assessed from results of triplicate analyses. Calibration curves were regularly generated to control instrumental drift. For all instruments used for analysis, the manufacturers’ operating procedures were strictly followed. All laboratory glassware used for analysis were washed with solution of HNO₃, rinsed twice with deionized water and placed in a clean environment until dry. Structured questionnaires were validated by administering randomly selected individuals in the study areas. During data collection, the consistency and accuracy of the questionnaires were checked regularly.

The limit of detection (LOD) of the method was determined from measurement of three blank samples that were digested and analyzed along with the samples. The LOD values were calculated as three times the standard deviation of the blank signals divided by the slope of the calibration curve, and were below 0.20 µg/g (Table 1) for all of the analyzed trace elements. Analysis of the potentially toxic elements were conducted by taking account the method detection limit of each metal.

3. Results and discussion

3.1. Demographic and lifestyle characteristics of the study participants

There were no significant differences between the two Sub-Cities, in terms of demographic and lifestyle characteristics of the participants (Table 2). Hence, this minimizes any interference from confounding factors on the measured chemical concentrations in the biological samples.

3.2. Proportion of samples with urinary 8-OHdG

Almost all of the urine samples (96.8 %) from the 95 individuals across the two Sub-Cities contained a detectable quantity, or greater, of 8-OHdG. However, in urine samples from 3 individuals, the level of 8-OHdG was below the LOD of the method. This indicates the presence of some level of DNA damage, and hence exposure to genotoxic substances.
3.3. Comparison of urinary 8-OHdG and nail heavy metals between Sub-Cities

The health status of the downstream (Akaki-Kality) residents compared with upstream (Gullele) residents of Addis Ababa were evaluated using urinary levels of 8-OHdG. Urinary 8-OHdG is commonly expressed as a ratio of the Creatinine levels of the urine, to eliminate urine dilution effect and control variations in urine flow rate [47]. In Table 3, the concentration of 8-OHdG is expressed as both the original concentrations in urine samples from residents of Akaki-Kality and Gullele Sub-Cities of Addis Ababa. Whiskers are 95 % confidence limits. Data symbolized by small circles are outliers and asterisks are extreme values.

Regarding the trace elements determined in nail samples, statistical analysis revealed that the concentrations of Fe, Zn, Cu, Mn and Pb differ significantly (p < 0.05) between residents of Akaki-Kality and Gullele. The nail samples from Gullele Sub-City contained significantly higher concentrations of these elements than those from Akaki-Kality. This is opposite of what was expected and disproves the expectation that urban pollution of the rivers drives metal exposure, in this case. The higher mean levels of Fe, Zn, Cu, and Mn from the upstream area, with no significant industrial or agricultural activities, may be attributed to other sources, such as exposure via drinking water and food that reflects differences in the geology and soil properties between the two Sub-Cities. Supporting this, as indicated in the Supplementary material – Appendix A, previous studies have found higher concentrations of these elements in the tap water and irrigation water from the upstream part of the catchment than the downstream part of the city in Akaki-Kality.

Table 3

| Sub-City | 8-OHdG (ng/mL) | 8-OHdG (ng/mg) | Fe | Zn | Cu | Mn | Ni | Cr | Pb | As |
|----------|----------------|----------------|----|----|----|----|----|----|----|----|
| Gullele (n = 42) | Median 19.8 | 18.0 | 685 | 245 | 6.01 | 23.5 | 1.42 | 1.40 | 0.54 | < LOD* |
| | Mean 21.3 | 17.3 | 1035 | 251 | 6.63 | 31.2 | 7.39 | 2.02 | 0.63 | 0.25 |
| | SD 13.0 | 5.83 | 875 | 64.8 | 2.66 | 23.3 | 11.3 | 2.01 | 0.52 | 0.45 |
| Akaki-Kality (n = 53) | Median 18.1 | 18.6 | 419 | 97 | 4.49 | 10.1 | 1.42 | 1.39 | < LOD | < LOD |
| | Mean 22.4 | 18.5 | 488 | 106 | 5.23 | 13.0 | 11.2 | 2.23 | 0.09 | 0.16 |
| | SD 16.0 | 4.37 | 357 | 72.8 | 2.26 | 11.0 | 58.1 | 2.48 | 0.22 | 0.29 |
| p value | 0.93 | 0.59 | 0.00 | 0.00 | 0.00 | 0.43 | 0.67 | 0.00 | 0.48 |

* < LOD is below limit of detection.
3.4. Demographic variations of urinary 8-OHdG levels

The measured 8-OHdG levels were not normally distributed, and hence non-parametric Kruskal-Wallis one-way ANOVA was used to evaluate the presence of significant differences in the urinary 8-OHdG levels among the different demographic groups. There were no statistically significant (p = 0.05) differences among the different demographic groups for gender, age, educational status, body mass index (BMI) or habit of alcohol consumption (Table 4).

The effects of the interactions between the different demographic characteristics were evaluated using two-factor ANOVA. The interactions of sex with age (p = 0.029), sex with alcohol consumption (p = 0.012) and alcohol consumption with education (p = 0.036) were found to affect the urinary 8-OHdG levels of residents of the two Sub-Cities. For example, interactions of sex with age, both young age females and males have higher amounts of 8-OHdG in their urines (Fig. 4A). In contrast, males tended to have higher amounts of urinary 8-OHdG in older ages than their female counterparts. The interaction of alcohol consumption and education also affected urinary 8-OHdG levels (Fig. 4B). Alcohol consumers who are illiterate tended to contain higher urinary 8-OHdG, and hence higher oxidative DNA damage, than non-consumer illiterates.

Many confounding factors, such as age, gender, exercise, alcohol, smoking, weight, and diet, may affect the 8-OHdG level, resulting in a higher degree of variability in results obtained from human subjects [48, 49]. However, data are usually inconsistent and vary from study to study. Locatelli et al. [50] investigated factors which influence the excretion of 8-OHdG in 24 h urine from 83 randomly selected healthy subjects; their results showed that smoking, body mass index (BMI), and gender were significant predictors of 8-OHdG excretion, while age was not [50]. According to Lin et al. [51], urinary 8-OHdG concentration was not affected by smoking or alcohol consumption, but was inversely related to age. Furthermore, Burgess et al. [52] conducted a study on non-smoking adults living in two Arizona communities and four Sonora, Mexico communities. Their research indicated no correlation between age and urinary 8-OHdG levels in Sonora, with or without Creatinine adjustment. BMI was significantly negatively correlated with Creatinine-adjusted urinary 8-OHdG in Sonora. However, in Arizona, BMI was positively correlated with Creatinine-adjusted urinary 8-OHdG, indicating that a single factor affects urinary 8-OHdG level differently.

In relation to sex [31] no significant difference was found in urinary 8-OHdG levels between male and female subjects (p = 0.1). Similarly, a study on adults exposed to heavy metals conducted by Wang et al. [30] found no significant difference in urinary 8-OHdG levels between different sex groups. On the other hand, Wu et al. [53] demonstrated that the normal value of 8-OHdG between males and females is significantly different (p = 0.001).

### Table 4

Comparison of average urinary 8-OHdG concentrations among demographic groups of residents of Akaki-Kality and Gullele.

| Demographic characteristics | 8-OHdG/UCr (ng/mg) | p value |
|----------------------------|-------------------|---------|
| Sex                        |                   |         |
| Female (n = 59)            | 17.6 ± 4.7        | 0.315   |
| Male (n = 36)              | 18.6 ± 5.4        |         |
| Age                        |                   |         |
| Young age adults (n = 43)   | 18.0 ± 3.2        | 0.979   |
| Middle age adults (n = 38) | 17.8 ± 5.2        |         |
| Old age adults (n = 14)     | 18.1 ± 6.3        |         |
| BMI                        |                   |         |
| Underweight (n = 3)        | 20.2 ± 1.0        | 0.640   |
| Middleweight (n = 71)      | 17.7 ± 5.2        |         |
| Overweight (n = 21)        | 18.4 ± 4.3        |         |
| Education                  |                   |         |
| Illiterate (n = 35)        | 18.1 ± 6.2        | 0.967   |
| Primary-Secondary          | 17.9 ± 4.5        |         |
| (n = 49)                   |                   |         |
| College Diploma            | 17.8 ± 2.0        |         |
| (n = 11)                   |                   |         |
| Alcohol consumption        |                   |         |
| Consumer (n = 28)          | 19.1 ± 5.1        | 0.166   |
| Non-consumer (n = 67)      | 17.5 ± 4.9        |         |

*Young age adults = aged between 18 and 35 years, Middle age adults = aged between 36 and 55 years, Old age adults = aged > 55 years, Underweight = BMI < 18.5, Middleweight = BMI in the range 18.5–24.9 and Overweight = BMI > 24.9.

3.5. Correlation analysis

Pearson correlation analysis was used to identify the presence of significant association between urinary 8-OHdG and trace element levels in nails of Akaki-Kality and Gullele residents (Table 5). In this study, no significant association between 8-OHdG and trace element levels was found, in either of the two Sub-Cities. Though not significant at p = 0.05, positive association (r = 0.053) was observed between urinary 8-OHdG and concentration of Mn in nail. Fe, Zn, Cu and Ni showed non-significant negative correlation with urinary 8-OHdG concentrations among residents of both Sub-Cities. They are essential elements that are crucial as antioxidants, and their deficiency has been correlated with increased markers of oxidative damage [54]. This might explain the observed negative correlation of these elements with urinary 8-OHdG level in this study. Furthermore, Pb and As, which are not essential elements, showed negative association and this suggests that an inverse association was observed between nail measures of Pb and As with measurable urinary 8-OHdG. Hence, the observed urinary 8-OHdG might be caused by other toxic chemicals in the environment that can cause oxidative DNA damage.

The absence of correlation was also confirmed with principal component analysis (PCA). Application of PCA provided four
components, each with Eigen value greater than one. The first two components together explained 50% of the variation in the data. As shown in Fig. 5A, residents of Akaki-Kality and Gullele, tend to differ in their nail heavy metal concentrations. Akaki-Kality occupy more of the negative side, while Gullele the positive side of PC1. Nail samples from Gullele are associated more with Fe, Mn, Zn and Cu (Fig. 5B). On the other hand, the urinary concentration of 8-OHdG, has shown neither discrimination between the two Sub-Cities nor association with any of the heavy metals, as it occupies the space far to the positive side of PC2.

3.6. Comparison of the measured urinary 8-OHdG levels with previous studies

The mean and median of urinary 8-OHdG levels measured in this study are generally higher than most of the values reported for various groups of people under different conditions and from different countries (Table 6). The measured concentrations are even higher than that reported for municipal waste incinerator workers and people occupationally exposed to chromium in China [55,56]. On the other hand, research conducted by Pizzino et al. in Italy [57] and Kim et al. in USA [58] have found urinary levels of 8-OHdG three to six times higher than the urinary levels of 8-OHdG measured in this study. A possible reason for these differences in environmental and occupational exposure to toxicants, as well as lifestyle [59], that can increase oxidative stress and in turn relates with urinary levels of 8-OHdG [60]. The higher urinary levels were reported for pregnant women from USA due to increased oxidative stress during pregnancy [61].

Most studies have reported the presence or absence of association between urinary 8-OHdG and the trace element content of urine (Table 6). Whereas, literature information on the link between the trace element content of human nail and urinary 8-OHdG is scarce. Much literature reports higher concentrations of 8-OHdG have been indicated in urine from people occupationally or environmentally exposed to various trace elements. The information available in the literature is also contradictory in many senses. For example, urinary 8-OHdG in concentrations of mean 15.2 ng/mg Creatinine, measured in adults from Japan [62], and median 11.7 ng/mg Creatinine, measured in children from Taiwan [63], has been associated with As and Cr in urine. Also, a lower median concentration of 5.04 ng/mg Creatinine has also been associated with Ni and Cu measured in urine of children with attention deficit hyperactivity disorder from China [64]. On the other hand, [65] have found no association between urinary 8-OHdG, with mean concentration of 7.17 ng/mL, and relatively high concentrations of various trace elements in the urines of municipal waste incinerator workers in China. Whereas, Bai et al. [56] have found association between urinary 8-OHdG, with mean concentration of 5.65 ng/mL, with Cr measured in the bloods of people exposed to chromate in China.

3.7. Binary logistic regression analysis

Binary logistic regression was applied, after adjusting for weight, education, BMI, alcohol consumption, location, age and sex, to understand the effects of heavy metals on the likelihood that participants have high levels of 8-OHdG biomarker. The dependent variable (8-OHdG level) was divided into two groups (≥ median and < median), depending on the median value. Dummy variables were created before running the binary logistic regression for the dependent variable i.e., 8-OHdG concentration. The independent variables were Fe, Zn, Cu, Mn, Ni, Cr, Pb and As levels in the nail samples from all 95 participants as well as weight, education, BMI, alcohol consumption, location, age and sex. As indicated in Table 7, the logistic regression model was statistically significant and fits the data, \( \chi^2(8) = 15.19, p = 0.02 \). The model explained 37% variation in the dependent variable (8-OHdG concentration), depending on Nagelkerke \( R^2 \) method and correctly classified 72.6% of cases. The results revealed that all the analyzed elements in human nails were not significant predictors for 8-OHdG concentration in urine samples. In addition, location, alcohol consumption, education, BMI, sex and age were not significant predictors of 8-OHdG. As presented in Table 7, B values for the logistic regression equation for predicting the dependent variable from the independent variable are negative for Zn, Cu and Ni, indicate that an inverse association was observed between nail measures of these with measurable urinary 8-OHdG. This suggests that the observed urinary 8-OHdG might be caused by other toxic chemicals in the environment that can cause oxidative DNA damage. Generally, the results of the binary logistic regression analysis were in agreement with previous findings.
Table 6
Comparison of the results of this study with data from literature.

| Country            | Age group            | Sample      | Analyzed metals                      | Element associated with 8-OHdG<sup>a</sup> | 8-OHdG/Cr (ng/mg) Ref. |
|--------------------|----------------------|-------------|--------------------------------------|-------------------------------------------|------------------------|
| Taiwan             | Male welders         | Urine       | Geometric mean (ng/mg Creatinine)     | Cr, Ni                                    | 4.77 [11]              |
|                    |                      | Urine       | Cr (2.06), Ni (3.13), Pb (3.13)       |                                           |                        |
| Taiwan             | Male office workers  | Urine       | Geometric mean (ng/mg Creatinine)     | –                                         | 2.54 [2]               |
|                    |                      | Cr (0.74), Ni (1.66), Pb (2.86) |                                           |                                           |                        |
| Taiwan             | Male adults          | Urine       | Geometric mean (ng/mg Creatinine)     | As                                        | 49.6 [51]              |
|                    |                      | Cr (282 ± 465), Mn (3.81 ± 11.4), Ni (81.5 ± 139), Pb (18.2 ± 49.6) |                                           |                                           |                        |
| China              | Exposed              | Blood       | Mean (ng/mL) Cr (5.75 ± 2.06)         | Cr                                        | 5.65 ± 2.86 [56]       |
|                    |                      | Mean (ng/mL) Cr (4.50 ± 2.23) | –                                     | Mean (ng/mL)                             | 4.76 ± 1.90 [57]       |
| Italy Adolescents  | Exposed              | Urine       | Geometric mean (ng/mL) Cr (1.52), Ni (0.65), As (2.69) | As                                        | –                      |
|                    |                      | Mean (ng/mL) Cr (0.74), Ni (1.66), Pb (2.86) | –                                     | Geometric mean (ng/mL) | 71.5                  |
| USA                | Pregnant Women       | Urine       | Geometric mean (ng/mL) Cr (1.24), Ni (0.27), As (1.38) | Cu                                        | 61.9 [58]              |
| Japan              | Adults               | Urine       | Mean (ng/mg Creatinine) Cr (0.12 ± 0.10), Cu (0.17 ± 0.16), Ni (0.02 ± 0.03), Pb (0.52 ± 0.41), Zn (0.52 ± 1.02) | As, Cr                                   | 15.2 ± 5.71 [62]       |
| Taiwan             | Children             | Urine       | Mean (ng/mL) Cr (1.91 ± 0.11), Ni (4.0 ± 0.2) | As, Cr                                   | –                      |
| China              | Cases (ADHD Children)| Urine       | Median (ng/mg Creatinine) Cr (1.48), Mn (1.09), Ni (4.0), Cu (16.6), Pb (2.64) | Ni, Cu                                   | 5.04 [64]              |
|                    | Controls             | Median (ng/mg Creatinine) Cr (0.90), Mn (0.66), Ni (2.08), Cu (11), Pb (0.88) | –                                     | Median (ng/mg Creatinine) | 4.09                  |
| China              | Exposed              | Urine       | Mean (ng/mL) Zn (713), Mn (69.6), Fe (604), Cr (123), Cu (25.3), As (62.4), Pb (3.2) | –                                        | 7.17 [65]              |
|                    | Controls             | Mean (ng/mL) Zn (766), Mn (5.80), Fe (78.8), Cr (4.46), Cu (40.8), As (48.8), Pb (4.69) | –                                     | Mean (ng/mL)            | 3.41                  |
| Ethiopia           | Akaki-Kality residents - adults | Nail | Mean (ng/mg) Fe (488 ± 357), Zn (106 ± 73), Cu (5.23 ± 2.26), Mn (13.0 ± 11), Ni (11.2 ± 58), Cr (2.23 ± 2.48), Pb (0.09 ± 0.22), As (0.16 ± 0.29) | –                                        | 18.5 ± 4.37 This study |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 17.3 ± 5.83 |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 17.5 |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 18.0 |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 19.7 |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 19.1 |
|                    |                      | Mean (ng/mg) Fe (1035 ± 875), Zn (251 ± 65), Cu (6.63 ± 2.66), Mn (31.2 ± 23.3), Ni (7.39 ± 11.3), Cr (2.02 ± 2.01), Pb (0.63 ± 0.52), As (0.25 ± 0.45) | –                                        | Mean (ng/mg Creatinine) | 19.8 |

<sup>a</sup> Sample type used for elemental analysis.

<sup>b</sup> Element that showed significant association with urinary 8-OHdG at p < 0.05.
good agreement with Pearson correlation analysis (Table 5) and principal component analysis (Fig. 5B).

4. Conclusions

In this study, the concentrations of 8-OHdG in urine and trace elements in nails from residents of Akaki-Kality and Gullele, the most downstream and upstream part of Addis Ababa, respectively, were determined. Contrary to the initial hypothesis, no statistically significant correlation was found between 8-OHdG levels and various demographic characteristics such as educational status, alcohol consumption, sex, age and body weight. However, the interactions of sex with age, sex with alcohol consumption and alcohol consumption with education were found to affect the urinary 8-OHdG levels of residents of the two Sub-Cities. Furthermore, none of the trace elements (Fe, Zn, Ni, Cr and Pb) analyzed in nails could be identified as a cause of the observed urinary 8-OHdG levels. In this study, we have not attempted to measure the impact of presence of 8-OHdG on human health outcomes. Presence of a substance does not necessarily imply that disease is inevitable and since many of the diseases caused by oxidative stress are chronic and take many years to manifest, an alternative study design would be required to evaluate risk to health such as a retrospective case control study or cohort study if prospective measurements were to be taken. Such a study is outside the remit of this project, but may be warranted in the future.

CRediT authorship contribution statement

Bitew K. Dessie: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Writing – review & editing. Bewketu Mehari, Molla Tefera, Sirak Robele Gari, Adey F. Desta, Samuel Melaku, Tena Alamirew and Michaela L. Goodson: Conceptualization, Writing – review & editing. Mahlet Osman and Yosef Tsegaye: Software, Data curation. Claire I. Walsh, Adane Mihret and Gete Zeleke: Conceptualization, Writing – review & editing, Supervision, Project administration, Resources, Supervision, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Author statement

As corresponding author, I confirm that the manuscript has been read and approved for resubmission by all the named authors. Consent from participating subjects was received prior to conducting the study. In addition, the study has been reviewed and approved by an accredited committee of Addis Ababa University, Ethiopia.

Table 7
Factors associated with the concentration of 8-OHdG (adjusted) in urine samples.

| Variables | R² | χ² | CP (%) | B  | p   | Exp(B) | 95 % CI for EXP(B) |
|-----------|----|----|--------|----|-----|--------|-------------------|
| Fe        | 0.37 | 8  | 0.02   | 72.6 | 0.00 | 1.00 | 0.99 | 1.00 |
| Zn        | 0.01 | 0.89 | 0.99 | 0.99 | 1.01 |
| Cu        | 0.06 | 0.64 | 0.94 | 0.72 | 1.22 |
| Mn        | 0.00 | 0.99 | 1.00 | 0.95 | 1.05 |
| Ni        | -0.05 | 0.20 | 0.95 | 0.89 | 1.03 |
| Cr        | 0.07 | 0.57 | 1.07 | 0.85 | 1.34 |
| Pb        | 0.75 | 0.30 | 1.97 | 0.84 | 8.78 |
| As        | 0.68 | 0.38 | 1.98 | 0.38 | 10.31 |
| Sub-City  | 0.68 | 0.42 | 1.98 | 0.38 | 10.31 |
| Alcohol   | 0.01 | 0.99 | 1.01 | 0.26 | 3.97 |
| Sex       | 0.16 | 0.79 | 1.18 | 0.35 | 3.93 |
| Education | -0.92 | 0.29 | 0.40 | 0.07 | 2.15 |
| BMI       | 0.50 | 0.39 | 1.66 | 0.52 | 5.26 |
| Age       | 1.04 | 0.23 | 2.85 | 0.54 | 15.40 |
| Constant  | 0.31 | 0.77 | 1.37 | –    | –    |

CP = Classification percentage of cases; reference category during logistic analysis were Gullele Sub-City, drunker, male, illiterate, underweight and young age adults.

* Hosmer and Lemeshow Test.
