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ORIGINAL PAPER

Use of toenail-bounded heavy metals to characterize occupational exposure and oxidative stress in workers of waterpipe/cigarette cafés

Mohammad Reza Masjedi · Sina Dobaradaran · Saeed Keshmiri · Farhad Taghizadeh · Hossein Arfaeinia · Farzad Fanaei · Mojtaba Behroozi · Farzaneh Nasrzadeh · Melika Joukar

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Abstract Tobacco smoke is known for releasing metals in indoor air of waterpipe/cigarette cafés. However, the worker exposure to metals, and its association with oxidative stress in these cafés are still unclear. To this end, 54 workers and 38 customers from waterpipe/cigarette cafés (the exposed group), 30 workers from non-smoking cafés (the control group 1 (CG_1)) and 32 individuals from the general population (the control group 2 (CG_2)) were selected and toenails samples were then taken from them. Our findings revealed a significant difference in terms of toenail-bounded metal levels between the exposure and control groups (CG_1 and CG_2) (Mann–Whitney U test, $P_{\text{value}} < 0.05$). This study has also indicated that "type of tobacco" could be considered as a predictor for toenail-bounded heavy metals. Furthermore, our research's results suggest that toenail-bounded heavy metals are positively and significantly correlated with urinary levels of 8- hydroxy-2′-deoxyguanosine (8-OHdG, as a biomarker for the degradation of deoxyribonucleic acid (DNA) oxidative stress). Therefore, it can be concluded that workers of waterpipe/cigarette cafés are at high risks of adverse health of DNA oxidative degradation.

Keywords Smoking · Waterpipe · Cigarette · Heavy metal · Toenail · DNA oxidative stress

M. R. Masjedi
Tobacco Control Research Center (TCRC), Iranian Anti-Tobacco Association, Tehran, Iran

S. Dobaradaran · S. Keshmiri · H. Arfaeinia · M. Behroozi · F. Nasrzadeh · M. Joukar
Systems Environmental Health and Energy Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran

S. Dobaradaran · H. Arfaeinia (✉)
Department of Environmental Health Engineering, Faculty of Health and Nutrition, Bushehr University of Medical Sciences, Bushehr, Iran
e-mail: arfaeiniah@yahoo.com

S. Keshmiri
Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran

F. Taghizadeh · F. Fanaei
Department of Environmental Health Engineering, Faculty of Health, Iran University of Medical Sciences, Tehran, Iran

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Introduction

In recent years, the consumption of waterpipe and cigarette has become more prevalent among teenager and young people in recent years (Asfar et al. 2005). In this regard, the use of waterpipe, as a wrong entertainment, had been prevalent for a long time in the Middle East, the Mediterranean, some South-American countries, and some Asian countries, including Iran, and it causes about 6 million (annually) deaths worldwide (WHO 2017). The health effects of tobacco are resulted from the interaction of the body with the hazardous and toxic substances found in tobacco smoke, which subsequently enter the human body through repeated puffs (Drago et al. 2018; Masjedi et al. 2019a). Tobacco smoke contains thousands of harmful and hazardous chemicals released into the air of cafés in the form of gaseous and particulate matter (Heydari et al. 2019; Rodgman and Perfetti 2016). Many scientific studies have focused on heavy metals present in the particle phase of the present species of cigarette smoke (Verma et al. 2010; Lugon-Moulin et al. 2004). Accordingly, it has been reported that various metals such as arsenic (As), lead (Pb), cadmium (Cd), manganese (Mn), and zinc (Zn) with considerable concentrations exist in tobacco smoke (Swami et al. 2009). Fromme et al. (Fromme et al. 2009) in their study have reported significant levels of carcinogenic metals such as lead (11.2 ng/m^3), cadmium (0.38 ng/m^3), and thallium (1.14 ng/m^3) in the indoor air inside of smoking cafés. As mentioned earlier, one of the major pollutants in tobacco smoke considered to be heavy metals whose its health effects have been well-documented. Accordingly, it was shown that the severity of these effects largely depends on the chemical state of the heavy metals (inorganic or organic), the metal's concentration, the contact time (duration of exposure), and the age of the smokers (Asante et al. 2012; Jafari et al. 2018; Haghnazari et al. 2018). Some heavy metals such as As, Pb, Cd, Ni, Cr, Co, and Hgare toxic elements that can cause several problems such as DNA damage, disruption of the hormonal balance, stimulation of the respiratory system, the decreased blood pressure, complications of the central nervous system, anemia, the decreasedintelligence quotient (IQ), the decreased physical and mental growth of children, the reduction of sperm production, leading to a disorder in activity of sperm'senzymes. Moreover, they can also cause some symptoms such as headaches, dizziness, abdominal pain, irritation, and skin ulcers (Ghezel-Ahmadi et al. 2010; Habibollahi et al. 2019). In addition, some heavy metals can be accumulated in the body, which consequently leads to damage to the kidneys and bone structure in case of a long time exposure of someone to tobacco smoke (Liu et al. 2020). Furthermore, they can cause cancer in the long-time exposure as well (Lim et al. 2019). Thus, human health might be threatened by metal exposure. In this regard, it is noteworthy that, the mechanism of metal-induced human diseases possibly involves oxidative stress (Zhang et al. 2019). Several animal and in vitro studies have revealed that redox active metals, such as Cu, Cr, Co, and other metals, undergo redox cycling reactions and produce reactive oxygen species (ROS), which overwhelms body antioxidant protection and subsequently induce DNA damage (Ni et al. 2014). Thus, it can be concluded that workers in tobacco cafés (waterpipe and cigarettes) are an important occupational group who are potentially exposed to heavy metals. However, there is no available data on the association between human metal exposure and DNA damage in these cafés.

Biomonitoring, as an appropriate approach, is widely used to assess occupational and environmental exposure to various pollutants (Yang et al. 2019; Camargo et al. 2019). In this regard, performing biological monitoring of heavy metals in different biological samples (such as nails, blood plasma, urine, and saliva) can indicate the exposure to heavy metals (Al-Saleh 2020; Camargo et al. 2019). It is noteworthy that the shelf-life of heavy metals in blood and urine is between 2 and 3 h and between 3 and 4 days, respectively. So, they can be considered them more suitable samples for assessing the acute exposure, while nails with a growth rate of 0.05 to 1.2 mm/week reflect a longer-term exposure (Ab Razak et al. 2015). Moreover, nails have many advantages such as non-invasive collection, easier collection and transportation, a higher stability at room temperature, and relatively higher concentrations of elements (metals) compared to the other biological specimens (such as blood, urine, sweat and other available tissues) (Li et al. 2012; Samanta et al. 2004). In addition, metals bounded to the nails are not exposed to the additional metabolic processes and many metals in considerably higher levels exist in the nail compared to blood plasma or urine samples (Hordinsky et al. 2000).

Toenails have less growth rate than finger-nails, which they also are less exposed to external contamination (Karatela et al. 2018). Therefore, toenail samples can represent the integrated heavy metal exposure over the past 2 to 12 months before the clipping (Karatela et al. 2018). So, the level of contaminants in toenails can be used as an indicator for monitoring as well as evaluating a long-term exposure to heavy metals in different occupational groups (Wongsasuluk et al. 2018).

However, to the best of our knowledge, no studies have been conducted on investigating heavy metals in toenails of people working in waterpipe/cigarette cafés so far, in order to evaluate their exposure to heavy metals. Therefore, for the first time, this study was conducted to evaluate the exposure of waterpipe/cigarette cafés' employees to heavy metal by measuring the heavy metals in their toenails samples. Furthermore, the effects of lifestyle and occupational factors such as age, sex, body mass index (BMI), the use of tobacco, type of tobacco consumed, work time duration in cafe's, cafés distance from traffic, and the use of protective equipment were evaluated in the present study as well. Finally, urinary level of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was used as a biomarker to evaluate the association between workers' metal exposure and degradation of DNA oxidative stress.

Materials and methods

Research design and sampling sites' selection

In this study, smoking cafés in Bushehr city, Iran were firstly reviewed and then listed. Afterward, 54 smoking cafés (of 83 available smoking cafés) were randomly chosen. To this end, smoking cafés were first coded from 1 to 83, and then 54 out of 83 cafés were randomly selected through formulation in Excel using the following randomization function (Eq. 1).

$$0 \leq 83 \times \text{Rand}\ () \leq 83 \quad (1)$$

Thereafter, addresses and other information of the selected cafés were provided for performing sampling. 34 out of 54 cafés were waterpipe cafés (hereafter referred to as WPC), and 20 ones were cigarette cafés (hereafter referred to as CC). One employee was selected from each café (totally 54 employee) and 38 subjects were selected from customers of these tobacco cafés. In addition, 30 non-smoking cafés were selected as the control group 1 (hereafter referred to as CG_1). Besides, a café worker and a customer were selected from each café; and toenail (all ten toes) samples were then taken from them.

Furthermore, 32 individuals from the general population were considered as the control group 2 (hereafter referred to as CG_2), and toenails samples were collected from them. It is noteworthy that people with any illness or disorder were excluded from the study to prevent its possible impact on the results. In this regard, all participants were healthy. After this step, the selected cafés were visited and then the necessary explanations were given to the cafés owners, managers, workers and customers to convince them, in order to obtain the sampling permit. Moreover, the smoking customers were aware that measurements would take place in indoor air of those cafés. It should be noted that this research was performed in terms of the ethical principles of Research Ethics Committee of Iran (IR.BPUMS.-REC.1399.012). The sampling operation began after convincing them as well as collecting informed consent forms.

Data collection and sampling

Prior to sampling, the background information of each café, such as the type of supplied tobacco (fruit flavored or traditional tobacco) and the cafés distance from traffic were recorded using the pre-designed checklist. In addition, sample donor information such as age, gender, height, weight, BMI, educational level, the use of tobacco, employment period, and the use of personal protective equipment were also recorded. The information on each café and each one of the studied people are given in Table 1. After performing this step, the included participants were taught about how to collect the samples correctly and the best way to prevent possible contamination was shown them. Therefore, a sample pack containing a disposable stainless steel razors and a polyethylene bag was given to the participants to ensure uniformity during sampling. The studied participants were then asked to wash their feet and toes with water and soap and to dry them with clean towels. Subsequently, they were asked to remove their toenails with a razors and to pack them in clean polyethylene bags. The samples

Table 1 General characteristics of participants

Characteristics	Exposed group		Control Group 1	Control Group 2
Subjects number	Employee (54.0)	Costumer (38.0)	30.0	32.0
Gender	Male (100%)	Male (100%)	Male (100%)	Male (100%)
Age (year)	34.0 ± 2.44	35.0 ± 2.29	32.0 ± 3.06	35.0 ± 2.56
Height (cm)	174 ± 4.12	178 ± 7.61	174 ± 6.43	176 ± 5.32
Weight (kg)	80.0 ± 7.87	79.0 ± 5.88	81.0 ± 6.45	79.0 ± 9.12
BMI (kg/m^2)	26.3 ± 1.09	26.4 ± 1.41	27.1 ± 1.12	25.5 ± 1.17
Education level (%)				
Illiterate/can only read and write	27.0	15.4	11.8	–
Up to 10 years of schooling	59.5	57.7	64.7	40.0
More than 10 years of schooling	13.5	26.9	23.5	60.0
Employment period (month)	29.7 ± 8.6	–	37.3 ± 46.7	–
Tobacco type (%)[a]				
Fruit flavored	29.7	34.4	–	–
Traditional	24.3	25.0	–	–
Cigarette	29.7	25.0	–	–
None	16.2	15.6	–	–
Traffic near the residence location (%)[b]				
Heavy	54.3	26.9	41.2	20.0
Moderate	25.7	53.8	29.4	35.0
Light	20.0	19.2	29.4	45.0
Personal protective equipment (%)				
No	81.1	–	00.0	–
Occasionally	13.5	–	23.5	–
Yes (full–time)	5.40	–	76.5	–

[a]The type of tobacco used by subjects (fruit flavored, traditional, cigarette, none)

[b]Traffic density in the district of each café

obtained from the control groups 1 and 2 were sampled in the same way. Moreover, customers of cafés were randomly sampled (their samples were also collected and then prepared as described earlier). Afterward, the collected toenails samples were immediately labeled, coded, and transferred to the laboratory. In the laboratory, the samples were packed appropriately and kept at room temperature for chemical analysis.

Sampler of this study had learned the necessary training to "transfer biological samples" and had extensive experience in appropriate handling (sampling, packaging and transporting) of biological samples such as urine, nails, and blood.

Preparation and analysis of nail samples

At this stage, the samples were thoroughly washed to remove any external contamination (Ndilila et al. 2014). The used method to clean the outer surface of the toenails was based on the published paper by Mehra et al. (Mehra and Juneja 2005). The toenail samples were then autoclaved for 45 min to eliminate any microbial contamination. Afterward, these samples were placed in Milli-Q water for 30 min in ultrasonic. Acetone was then replaced with Milli-Q water and it was again placed in ultrasonic for another 30 min, to remove any organic contamination (Ndilila et al. 2014). Next, the toenails were rinsed 5 times with Milli-Q water and then dried at oven for 60 min until their weight became a fixed. Thereafter, these dried toenails samples were digested using wet acid digestion method (4 ml concentrated nitric acid) at 65 °C for 2 h. Then, 4 ml of hydrogen peroxide was added to the samples and held at 65 °C until the time that reaction was complete. After preparing the samples, a quadrupole Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP) equipped with Mass Spectrometry (MS) (ICP-MS, Elan 5000, Perkin Elmer) apparatus was used to quantify the concentration of 8 heavy metals including Pb, Cd, Co, As, Cr, Ni, Zn and Cu. The parameters for ICP-MS are as follows: forward power of 1000 W, nebulizer gas flow rate of 0.8 L/min, sample injection mode: manual mode, integration time: 5 s, and sampling depth: 10 mm.

For quality control and quality assurance (QC/QA), parallel to the samples preparations, the blank samples were also extracted with a similar procedure without toenail samples. Multi-element standard solution was purchased from Merck (Darmstadt, Germany) and utilized for preparation on calibration standards. Moreover, the equipment was calibrated on a daily basis by calibration standards. A spiked blank, method blank and matrix spike, as well as replication of the samples were performed for each batch of the samples. All samples were analyzed in triplicate to demonstrate reproducibility of the equipment. The relative deviation of the replicate samples was within ± 5% in all batch runs. The extent of recovery for the 8 metals in the spiked blank samples was obtained within the range of 76.4–113% with a standard deviation of < 10%. Further, in the samples, for 8 metals in matrix spike samples, it was achieved within the range of 80.41–111% with a standard deviation of about 10.3%. Very little levels of some elements were seen in the method blank samples, which were suitably subtracted from the values read in the samples. The correlation coefficients (R^2) and linear ranges were 0.1–99.97 ng/mL and 0.9995 for Zn, 0.1–19.97 ng/mL and 0.9994 for Cu, 0.1–9.77 ng/mL and 0.9983 for Pb, 0.1–9.74 ng/mL and 0.9974 for Cd, 0.5–10.17 ng/mL and 0.9994 for As, 0.1–9.73 ng/mL and 0.9963 for Co, 0.1–9.97 ng/mL and 0.9998 for Cr, and 0.1–9.69 ng/mL and 0.9967 for Ni. Correlation coefficients (R^2) ranging from 0.9963 to 0.9998 indicated good linearity for all metals. The method limits of detection (LODs) were 0.017 μg/g for Zn, 0.011 μg/g for Cu, 0.006 μg/g for Pb, 0.009 μg/g for Cd, 0.014 μg/g for As, 0.007 μg/g for Co, 0.017 μg/g for Cr, and 0.018 μg/g for Ni.

Analysis urinary 8-OHdG

A subset of 134 urine samples collected from waterpipe/cigarette cafés (54 workers and 38 customers), the control group 1 ($n = 30$) and control group 2 ($n = 32$) were analyzed using the 8-OHdG Check ELISA kit (Japan Institute for the Control of Aging, Nikken SEILCo., Shizuoka, Japan) for 8-hydroxydeoxyguanosine (OHdG), which is a product of oxidative DNA damage formed by hydroxyl radicals. The coefficient of variation (CV) and the recovery rates of 8-OHdG was ranged from 7.21 to 8.47% and 94.0–113%, respectively. Details regarding the sample preparation and analysis of 8-OHdG are described elsewhere (Bamai et al. 2019).

Statistical analyses

Statistical analyses of the current research were performed using Microsoft Excel 2016 and SPSS (version 21.0; SPSS Inc.). The latest version of R software (R 4.0.2 for Windows) was utilized to prepare the box and whisker plot. Moreover, Shapiro–Wilk test was used to evaluate the data distribution normality for each of the variables. Difference between toenail metals levels between exposed and control groups were examined by Mann–Whitney *U* nonparametric test. Since the data distribution of metals was not normal, so the metal concentrations were analyzed by the log-transform multiple linear regression analysis. Accordingly, this analysis was done by the association between concentrations of metals and independent variables, including age, BMI, tobacco use, type of tobacco (fruit-flavored tobacco, traditional tobacco, and cigarette), distance from traffic, length of time spent in the cafés, and use of personal protective equipment. $P_{\text{value}} < 0.05$ was considered as the statistical significant level and all P_{value}s in this research were two-sided.

Results and discussion

Demographic and lifestyle characteristics of participants

In this study, a group of 92 individuals (54 workers and 38 customers) from smoking cafés were considered as an exposed group. Two non-exposed groups, including 30 non-smoking cafés workers (the control group 1 (GC_1)) and 32 subject from general populations (control group 2 (GC_2)) were selected. The demographic and lifestyle characteristics of these research subjects are presented in Table1 (based on the checklists' information). As shown, all the subjects were male with the mean age of 34, 35, 32, and 35 years in smoking cafés workers, customers, GC_1, and GC_2, respectively. The participants in GC_1 were slightly younger than the other two groups. There was no significant difference in terms of weight, height and BMI between the exposed and the control groups. According to the WHO classification, most of the subjects were in slightly overweight (58%) and normal weight (33%) classes; and 9% of participants were overweight. Moreover, most of the individuals of the exposed group had low socioeconomic and educational status. Notably, there was a significant difference (Mann–Whitney *U* test, *P* value < 0.05) in employment periods between smoking cafés workers and CG_1 group, in a way that non-smoking cafés workers (CG_1) had a longer employment duration. Most of these subjects used fruit-flavored tobacco. Unfortunately, a few people in the smoking cafés used personal protective equipment (about 5%).

Toenail metals concentrations in exposure/control groups

Toenails are considered as one of the body tissues, in which many intaked metals are accumulated. In addition, toenails metal levels could be used as a biological index to reflect the recent exposure (recent several days-weeks) (Ab Razak et al. 2015). In this study, the concentrations of 8 heavy metals (lead, cadmium, arsenic, nickel, chromium, cobalt, copper and zinc) were examined in the toenail samples collected from the exposed groups (including waterpipe/cigarette cafés workers and customers) and the two control groups (CG_1 and CG_2).In this regard, the statistical results are presented in Fig. 1 and Table 2. Except As, Pb, Cd and Ni in the two control groups (CG_1 and CG_2), the rest of metals were detected ($>$ LOQ) in all 154 samples. The mean concentration of $\sum$heavy metal in the toenailsamples obtained from waterpipe cafés and cigarette cafés workers were 5.31 and 4.67 times higher than the CG_1 samples and 8.39 and 7.37 times higher than the CG_2 samples, respectively. Thus, it can be strongly

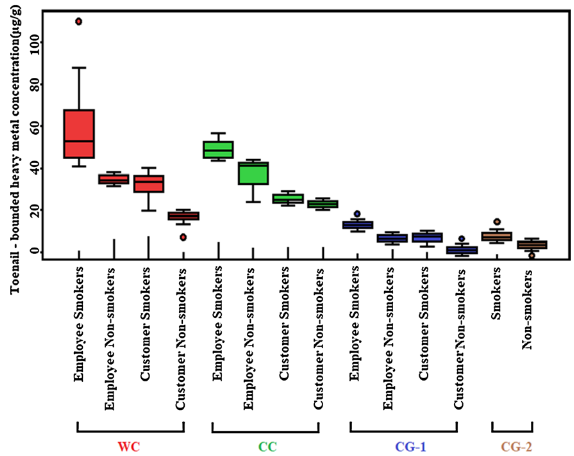

Fig. 1 Box plot of levels of toenail-bounded heavy metals (µg/g) in exposed and control groups (employee: workers in waterpipe/cigarette cafés, Customers: customers in waterpipe/cigarette cafés, CC: cigarette cafés, WC: waterpipe cafés, CG-1: control group 1 and CG-1: control group 2) (in this box plot a, b, c, d, e and f are outliers, maximums, upper quartiles, medianss, upper quartiles and minimums, respectively)

stated that working in smoking cafés was a considerable source of exposure to various pollutants like heavy metals (Swami et al. 2009). Moreover, it was found that the median concentrations of $\sum$heavy metal were higher in the toenail samples collected from waterpipe/cigarette cafés workers compared to the samples obtained from the customers of these cafés. The higher concentrations of toenail metals were also observed in smoking cafés workers compared to the customers, which might be due to the fact that cafés workers usually spend much more time in cafés with a low quality air; therefore, their exposure to tobacco smoke contaminants is higher (Akl et al. 2010; Gurung et al. 2016). The findings have also indicated that the amounts of metals were higher in waterpipe cafés workers and customers compared to cigarette cafés. The findings of our previous studies have also indicated that the concentrations of emitted metals were higher by waterpipe smoking compared to cigarette (Heydari et al. 2019; Masjedi et al. 2019b). Furthermore, there was a significant difference in terms of metal levels between the toenails samples of the exposure and control groups (CG_1 and CG_2) (Mann–Whitney *U* test, *P* value < 0.05). Consequently, this indicates that there was a close relationship between smoking activities and a higher accumulation of metal in the toenails. Numerous

Table 2 Statistical analysis of toenail heavy metal concentration among exposed/unexposed groups (μg/g)

Exposure type	Statistical analysis	Pb	Cd	As	Ni	Co	Cr	Cu	Zn	∑Metal
WPC										
Workers	Mean ± SD									
	(min–max)	1.87 ± 0.81 (0.18–3.54)	0.82 ± 0.53 (0.07–2.11)	0.48 ± 0.28 (0.09–1.11)	2.72 ± 1.54 (0.56–7.32)	1.41 ± 1.01 (0.06–4.05)	3.23 ± 2.81 (0.09–11.87)	13.41 ± 6.21 (2.32–23.32)	27.82 ± 10.48 (8.67–67.41)	51.73 ± 23.67 (12.04–120.73)
	Comparison with GC1	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
	Comparison with GC2	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Customers	Mean ± SD									
	(min–max)	0.94 ± 0.64 (0.12–2.78)	0.55 ± 0.38 (0.09–1.23)	0.21 ± 0.17 (0.07–0.81)	1.52 ± 1.77 (0.19–6.16)	0.79 ± 0.63 (0.02–2.51)	1.94 ± 1.23 (0.11–4.58)	8.06 ± 6.27 (2.98–13.23)	12.95 ± 3.38 (3.24–22.17)	26.96 ± 14.64 (6.82–53.47)
	Comparison with GC1	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
	Comparison with GC2	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
CC										
Workers	Mean ± SD									
	(min–max)	1.66 ± 0.62 (0.19–2.75)	0.73 ± 0.28 (0.14–1.11)	0.41 ± 0.28 (0.12–0.89)	2.48 ± 1.75 (0.11–5.43)	1.31 ± 1.02 (0.05–3.25)	2.85 ± 3.05 (0.34–12.12)	11.67 ± 8.08 (1.67–34.32)	24.41 ± 9.81 (4.45–37.78)	45.52 ± 24.89 (7.07–97.65)
	Comparison with GC1	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
	Comparison with GC2	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Customers	Mean ± SD									
(min–max)	0.91 ± 0.53 (0.07–1.67)	0.45 ± 0.26 (0.09–0.88)	0.18 ± 0.20 (0.08–0.71)	1.31 ± 1.02 (0.09–3.32)	0.75 ± 0.68 (0.04–2.09)	1.78 ± 1.02 (0.43–3.67)	7.17 ± 4.16 (2.32–16.42)	11.81 ± 2.43 (8.76–16.55)	24.36 ± 10.50 (11.88–45.31)	
	Comparison with GC1	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
	Comparison with GC2	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
GC-1										
Workers										
	Mean ± SD									
	(min–max)	0.33 ± 0.24 (> LOD-0.89)	0.13 ± 0.07 (> LOD-0.33)	0.08 ± 0.08 (> LOD-0.39)	0.28 ± 0.25 (0.08–0.98)	0.15 ± 0 .23 (0.04–1.07)	0.39 ± 0.41 (0.09–1.67)	3.98 ± 3.42 (0.45–12.31)	4.41 ± 2.56 (0.09–11.22)	9.75 ± 7.26 (0.75–28.86)
	Comparison with GC2	$P = 0.67$	$P = 0.44$	$P = 0.78$	$P = 0.54$	$P = 0.67$	$P = 0.78$	$P = 0.74$	$P = 0.61$	$P = 0.58$

Table 2 continued

Exposure type	Statistical analysis	Pb	Cd	As	Ni	Co	Cr	Cu	Zn	$\sum$Metal
Customers	Mean ± SD (min–max)	0.16 ± 0.12 (> LOD-0.56)	0.098 ± 0.057 (> LOD-0.29)	0.048 ± 0.029 (> LOD-0.11)	0.18 ± 0.15 (> LOD-0.67)	0.12 ± 0.16 (0.02–0.73)	0.22 ± 0.25 (0.02–1.01)	1.89 ± 0.95 (0.67–4.32)	2.52 ± 2.69 (0.04–7.04)	5.23 ± 4.41 (0.75–14.73)
	Comparison with GC2	$P = 0.58$	$P = 0.76$	$P = 0.69$	$P = 0.23$	$P = 0.76$	$P = 0.81$	$P = 0.76$	$P = 0.71$	$P = 0.69$
GC-2	Mean ± SD (min–max)	0.17 ± 0.16 (> LOD-0.76)	0.068 ± 0.022 (> LOD-0.56)	0.043 ± 0.026 (> LOD-0.09)	0.15 ± 0.17 (> LOD-0.66)	0.14 ± 0.16 (0.02–0.61)	0.27 ± 0.21 (0.02–0.67)	1.87 ± 0.88 (0.78–4.88)	3.43 ± 2.37 (1.09–11.78)	6.16 ± 3.99 (1.91–20.01)

studies have found a positive association between toenails metal levels and occupational exposure (Hinwood et al. 2003; Grashow et al. 2014). In addition, for both exposed and control groups, subjects were classified into smokers and non-smokers and the concentrations of $\sum$heavy metal were compared in smokers and non-smokers (Fig. 1.). As can be observed, the median concentrations of $\sum$heavy metal in toenails collected from smoker subjects were higher than samples of non-smokers in all groups (WC, CC, CG_1 and CG_2) (P value < 0.05). The reason for this observations can be due to the fact that smoking is one of the main contributor to the body burden of heavy metal (Kim et al. 2010). Other studies have also confirmed that the levels of heavy metals (and other toxic pollutants) is higher in biological samples of smokers than non-smokers (Gregg et al. 2013; Rocha et al. 2016). The concentration of toenail-bound metals were higher in comparison with those reported for some industrial communities in Zambia (Ndilila et al. 2014). The findings of toenail-bounded metals were also higher than the corresponding values for the occupational welding fume exposure (Grashow et al. 2014; Laohaudomchok et al. 2011) and lower than those measured in Portuguese miners (Coelho et al. 2012). Higher toenail metal levels were recorded in a research from an industrialized area with high levels of environmental exposures from air particles and dust (Slotnick et al. 2005). However, those findings might have been skewed by a small number of extremely high exposures, and were estimated using adults and children, where children tend to have higher toenail metal concentrations than adults (Wilhelm et al. 1994; Wickre et al. 2004).

Geometric mean concentrations of Pb, Cd and As (three toxic metals of major public concern) in the toenails samples were 1.78, 0.82 and 0.48 μg/g in waterpipe cafés workers, 1.66, 0.77, 0.41 μg/g in cigarette cafés workers, and 0.33, 0.13, and 0.08 μg/g in non-smoking cafés workers (CG_1), respectively. Also, in the toenails samples from the cafés customers, geometric mean concentrations of three toxic metals (Pb, Cd, and As) were 0.94, 0.55 and 0.21 μg/g in waterpipe cafés customers; 0.91, 0.45, 0.18 μg/g in cigarette cafés customers, and 0.16, 0.098 and 0.048 μg/g in non-smoking cafés customers (CG_1). Furthermore, geometric mean concentrations of three toxic metals (Pb, Cd and As) in general population (CG_2) were measured as 0.17, 0.068 and 0.043 μg/g, respectively. Therefore, it can be concluded that tobacco smoke is an important source of toxic metals in indoor air of smoking cafés. So, people exposed to this smoke would consequently receive high concentrations of heavy metals. In agreement with this conclusion, Omari et al. reported high values of Pb in cigarette smoke of various brands in Kenya (Omari et al. 2015). Also, in a study by Fromme et al. (2009) a significant concentrations of carcinogenic metals, such as lead (11.2 ng/m^3), cadmium (0.38 ng/m^3), and thallium (1.14 ng/m^3) were reported in indoor air of the smoking cafés. Most of the heavy metals in tobacco smoke are originated from the tobacco leaves' burning. It is notable that tobacco is a fast-growing plant, which absorbs heavy metals present in the soil into its tissues similar to other natural trees (Pappas et al. 2007). Additionally, tobacco is a sensitive plant; therefore, it is prone to many diseases. So, the farmers must use various fertilizers, herbicides, and pesticides to protect these plants from pests (Zhao et al. 2020). These pesticides usually contain heavy metals in their structures that enter various parts of the tobacco plant, and then release through smoke while burning, and finally affect the exposed people (Sebiawu et al. 2014).

In general, given the high levels of heavy metals in the toenails of workers and customers in waterpipe/cigarette cafés, it can be claimed that these individuals are highly exposed to serious health risks. In line with this claim, some previous studies have reported an association between heavy metals exposures and a higher Peripheral Artery Disease (Arora et al. 2009); among smoking, exposure to heavy metals and pancreatic cancer (Schwartz and Reis 2000), as well as the association between smoking, exposure to heavy metals, and diabetes (Schwartz et al. 2003). Another study has also reported a relationship between smoking history and cadmium accumulation in the lung tissue (Pääkkö et al. 1989). It should be noted that the biological half-life of some metals is between 20–25 years, and they can have bioaccumulation in different parts of the smokers' body due to having a high biological half-life (Suwazono et al. 2009). People with a wide age range were present in these cafés. However, active smokers were generally young men and women who spent their leisure time with their friends and waterpipe smoke. In some cases, it was also observed that children and women were also present in these cafés. Therefore, raising the awareness of women and notification about consumption of tobacco products is essential. It is also suggested that kindergartens and children schools consider some training about the harms of waterpipe and tobacco to children and students.

Effect of lifestyle and occupational factors

The multiple regression analysis (MLR) was used to evaluate the association between the measured toenail-bounded metals with influential occupational and lifestyle variables, and the results are provided in Table 3. In this analysis, the correlation between metals concentration with these factors was evaluated based on the regression coefficient (β coefficient) and P value. As can be seen, type of tobacco, number of services per day, café's distance from traffic, and the use of personal protective equipment were identified as the predictors of toenail-bounded heavy metal concentrations. The other examined factors examined in this study including age, BMI, educational level, and duration of working in cafés had no significant relationship with the concentrations of toenail-bounded heavy metals.

There was a significant positive relationship (P_{value} < 0.01) between amounts of toenail-bounded heavy metals and type of tobacco. For further investigation, total toenail-bounded heavy metals ($\sum$toenail-bounded heavy metal) were compared in those working in cafés with fruit-flavored and traditional tobacco cafés and results are depicted in Fig. 2. As shown, total amounts of heavy metals in the toenail samples of those, who worked in cafés with fruit-flavored and traditional tobacco cafés, were 68.88 ± 20.49 and 39.57 ± 6.34 µg/g, respectively. For the customers, the participants' metal contents were 32.71 ± 7.12 and 20.52 ± 3.93 µg/g by consuming fruit-flavored and traditional tobacco, respectively. Fruit-flavored tobacco contains high levels of organic chemicals, aroma, essential oils, and flavoring additives that are added to this type of tobacco during its manufacturing process (Farley et al. 2018). Therefore, this type of tobacco releases higher amounts of various air pollutants into the indoor air of cafés during burning, and consequently, those who work in such cafés, are more exposed to higher concentrations of contaminants (Rostami et al. 2019; Heydari et al. 2019). In this regard, high concentrations of toenail-bounded heavy metals in workers of fruit-flavored tobacco cafés can be due to the above-mentioned reason. In line with

Table 3 Multivariate linear regression (MLR) analysis of toenail-bounded metal (μg/g) with factors affecting exposure to metal [β coefficient (P value)]

Affecting Factors	Zn	Cu	Pb	Cd	Ni	Co	Cr	As
Age (years)	0.101 (0.59)	0.056 (0.54)	0.088 (0.719)	0.081 (0.51)	0.085 (0.50)	0.135 (0.30)	0.071 (0.37)	0.122 (0.40)
BMI (kg/m^2)	− 0.011 (0.78)	− 0.015 (0.66)	− 0.009 (0.70)	− 0.010 (0.63)	− 0.013 (0.55)	− 0.020 (0.37)	− 0.009 (0.68)	− 0.014 (0.56)
Education level	− 0.071 (0.53)	− 0.026 (0.59)	− 0.019 (0.65)	− 0.022 (0.37)	− 0.011 (0.49)	− 0.021 (0.80)	− 0.009 (0.75)	− 0.111 (0.57)
No. of services per each working shift	0.498 (0.02)	0.503 (0.03)	0.225 (0.03)	0.395 (0.03)	0.301 (0.02)	0.188 (0.03)	0.365 (0.03)	0.201 (0.03)
Employment period (month)	0.161 (0.34)	0.197 (0.19)	0.157 (0.43)	0.134 (0.50)	0.112 (0.27)	0.113 (0.56)	0.113 (0.14)	0.145(0.30)
Tobacco type	0.651 (0.01)	0.283 (0.02)	0.703 (0.01)	0.767 (0.01)	0.309 (0.01)	0.392 (0.01)	0.470 (0.01)	0.545 (0.01)
Traffic near the residence location	0.465 (0.03)	0.203 (0.02)	0.366 (0.02)	0.293 (0.03)	0.324 (0.02)	0.302 (0.03)	0.177 (0.03)	0.221 (0.03)
Personal protective equipment	− 0.356 (0.02)	− 0.301 (0.02)	− 0.245 (0.03)	− 0.192 (0.03)	− 0.123 (0.08)	− 0.255 (0.03)	− 0.326 (0.02)	− 0.208 (0.03)

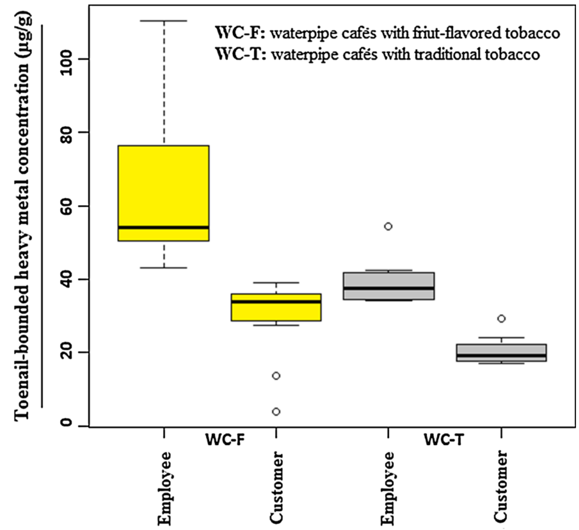

Fig. 2 Box plot of levels of toenail-bounded heavy metals (μg/g) stratified by "tobacco type" (Employee: workers in waterpipe/cigarette cafés, Customers: customers in waterpipe/cigarette cafés, WC-F: waterpipe cafés with fruit-flavored tobacco and WC-T: waterpipe cafés with traditional tobacco)

these statements, Naddafi et al. (2019) in their study reported higher values of PAHs, particles, formaldehyde, and acetaldehyde in the air inside the fruit-flavored tobacco cafés compared to the traditional tobacco cafés. Masjedi et al. (2019a) have also observed high values of gaseous pollutants such as CO, formaldehyde and nicotine inside the cafés that supplied the fruit-flavored tobacco. Because of the sweet and tasty flavor of this type of tobacco, and its slow burning, fruit-flavored tobacco is known as a fun activity for young people who tend to spend much time in these cafés. Therefore, they are exposed to higher concentrations of pollutants (Naddafi et al. 2019).

There was a positive and significant relationship ($P_{\text{value}} < 0.05$) between concentrations of toenail-bounded heavy metals with number of services per day and café's distance from traffic. Accordingly, number of services per day in cafés refers to number of "active waterpipe /cigarette heads" per day. Increasing number of "active waterpipe /cigarette heads" within cafés subsequently raises amounts of tobacco smoke in the indoor air, thereby producing higher concentrations of contaminants and more exposing the cafés workers and customers (Rostami et al. 2019; Heydari et al. 2019; Masjedi et al. 2019a). The traffic density of areas, where the study population lived, also was another factor affecting the measured toenail-bounded metals levels (Table 3). It was consistent with the findings of previous studies that reported the higher exposure to air contaminants for people living near the busy roads compared to those living in a far distance from roads (Jafari et al. 2018; Phuc and Kim Oanh 2018).

A significant but negative relationship was also observed between the use of personal protective equipment (N95 mask, etc.) and toenail-bounded metal concentrations ($P_{\text{value}} < 0.05$). The finding can be attributed to the efficiencies of filters and mask on preventing the entry of solid and liquid suspended particulates. Since most of the released heavy metals in the air are in the particulate phase (Ndilila et al. 2014), the use of appropriate masks and filters can reduce the individual exposure to these particulate pollutants as well as reducing their amounts (heavy metals) in toenail samples. Previous investigators have also demonstrated that demographic and lifestyle factors are important pathways and contributors for metals exposure (Ndilila et al. 2014). Age, resident period (exposure period), previous occupational exposure and passive cigarette smoke exposures have all been shown to be important factors influencing metals exposure (Hogervorst et al. 2007).

Metal exposures and oxidative stress

The level of 8-hydroxy-2′-deoxyguanosine (8-OHdG, as a biomarker of oxidative stress) was measured in the urine samples collected from the waterpipe/cigarette cafés workers and customers, and the control groups, to evaluate the relationship between heavy metal exposure and oxidative stress in them. Thereafter, the Pearson correlations were used to evaluate the association between the values of toenail-bounded heavy metals and the concentration of 8-OHdG, as presented in Table 4. As shown, the concentration of 8-OHdG was positively and significantly related to the concentrations of all toenail-metals among waterpipe/cigarette cafés workers and customers; however, the relationship was only for the concentrations of 8-OHdG and zinc in the control groups (CG_1 and CG_2). The findings suggested that the exposure to heavy metals was associated with oxidative degradation of DNA in the waterpipe/cigarette cafés workers and customers. Among all these metals, arsenic, cadmium, lead, and chromium had high correlation

Table 4 Pearson's correlation coefficients among toenail-bounded metals and 8-OHdG of workers working in waterpipe/cigarette cafés

Sampling Café	Zn	Cu	Pb	Cd	Ni	Co	Cr	As
WPC	0.723**	0.369*	0.475**	0.495**	0.403*	0.333*	0.456**	0.625**
CC	0.643**	0.331*	0.461**	0.482**	0.365*	0.302*	0.409*	0.552**
CG_1	0.467**	0.119	0.083	0.203	0.122	0.098	0.223	0.189
CG_2	0.482**	− 0.115	− 0.033	0.074	0.073	0.069	0.115	0.081

"**" represents $P < 0.01$; "*" represents $P < 0.05$

coefficients measured as 0.625, 0.495, 0.475 and 0.456 in waterpipe cafés and 0.552, 0.482, 0.461 and 0.409 in cigarette cafés, respectively. To the best of our knowledge, As, Cd, Pb, and Cr are known as toxic metals. The results of numerous epidemiological studies indicated that the exposure to the above-mentioned metals is associated with the increased oxidative degradation of DNA in humans (Wong et al. 2005; Wei et al. 2009; Ni et al. 2014). Findings of the present study are consistent with the results reported by Zhang et al. (2019), who studied the exposure to heavy metals in the individuals working in the electronic waste recycling facilities. An interesting finding of the present study was a positive and significant relationship found between the concentrations of 8-OHdG and zinc even in the control groups. Several in vivo and in vitro studies have indicated that essential elements, such as zinc, copper, and cobalt can also cause redox cycling reactions and also are capable of producing active species and free radicals such as nitric oxide and superoxide anion radical in biological systems like human body, so they have a high potential for health risks (Speisky et al. 2009; Galanis et al. 2009; Jomova and Valko 2011; Liochev and Fridovich 2000). Despite the fact that the mechanism of the increased oxidative stress by essential elements needs a more accurate examination by performing other studies, a significant positive association found between toenail-bounded metals and oxidative stress biomarkers in waterpipe/cigarette cafés workers and customers indicated that they are exposed to greater health risks.

Limitations and ideas for future researches

The current study had a series of limitations that should be widely taken into account in using the findings for smoking cafés elsewhere in the world. The first limitation of this research was a low sample size; therefore, further studies should be carried out in a more comprehensive way with greater sample sizes. Moreover, the investigated contaminants in this study were limited only to heavy metals, while some other dangerous and toxic pollutants in tobacco smoke such as propylene oxide, benzene, toluene, ethylbenzene and xylene (BTEX), acrolein, acrylonitrile, polycyclic aromatic hydrocarbons (PAHs), 1,3-butadiene, ethylene oxide, nitrosamines, and their metabolites were not investigated in the biological samples of the exposed people in this study due to a lack of sampling and analysis equipment as well as lack of research funding. So, they can be taken into account as attractive ideas for future studies. In addition, waterpipe and cigarettes smoke are not the only sources of exposure to heavy metals in such cafés, and some other sources of heavy metals that enter the body such as drinking water and foods can also be considered. Therefore, besides toenails samples, there is a need for performing a more comprehensive and detailed study on these metals in drinking water samples, foodstuffs in such cafés, and so on. Furthermore, a closer examination of the mechanism of oxidative stress increase caused by heavy metals in tobacco smoke requires more detailed evaluations.

Conclusion

Despite the fact that the current work was the first work conducted on levels of toenail-bounded heavy metals in waterpipe/cigarette cafés workers and customers have some limitations, it also had valuable results, indicating that their exposure to heavy metals was significantly high. Findings of the present study have also indicated that there was a significant difference in terms of the toenail-bounded metals between the exposed and the control groups. Moreover, the results confirmed that working in waterpipe/cigarette cafés is a potential occupational hazard in terms of exposure to heavy metals. The present study also indicated that “type of tobacco” could be used as a predictor for toenail-bounded heavy metal concentrations, in a way that those, who worked in cafés serving fruit-flavored tobacco or were customers, showed higher levels of heavy metals in their toenail samples. Furthermore, number of services per day, distance from traffic, and use of personal protective equipment were three predictors of concentrations of toenail-bounded heavy metals in waterpipe/cigarette cafés workers and customers. In addition, the results of the present study suggest that the concentrations of toenail-bounded heavy metals in the studied participants were significantly and positively correlated with the urinary concentration of 8-OHdG, which is a biomarker for degradation of DNA oxidative stress. Finally, it can be concluded that waterpipe/cigarette cafés workers are at a high health risk for oxidative degradation of DNA. Therefore, effective protective strategies are needed to reduce their exposure and amounts of various chemical pollutants in the air inside the cafés.

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